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(54) Title: TREATMENT OF HIV-1 INFECTION AND INFLAMMATORY DISEASE USING CYCLOPHILIN RECEPTOR ANTAGONISTS
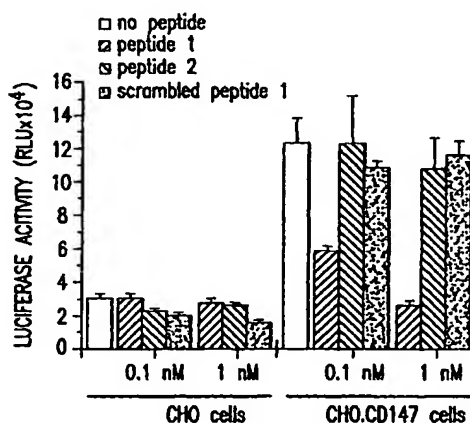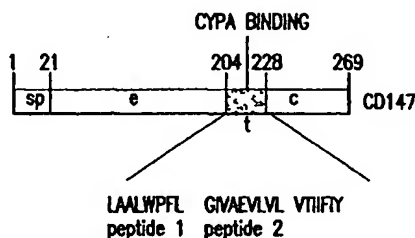
(57) Abstract: Examples and embodiments of the present invention teach and disclose, inter alia, that the previously known extracellular matrix metalloproteinase inducer protein (EMMPRIN), also known as CD147, is a signal-transducing cellular receptor for cyclophilin (CyP). Accordingly, there is disclosed methods for the treatment of HIV-infection, AIDS, AIDS-related disorders., rheumatoid arthritis (RA), connective tissue disorders, cancer or any condition characterized by local or systemic CyP release, synthesis or binding using the CyP/EMMPRIN interaction as a novel therapeutic intervention target. There is further disclosed pharmaceutical compositions including, anti-EMMPRIN antibodies, and soluble EMMPRIN proteins and peptides that act by inhibiting the CyP/EMMPRIN interaction. The present invention further provides screening assays for the identification of compounds that inhibit the CyP/EMMPRIN interaction.

# TREATMENT OF HIV-1 INFECTION AND INFLAMMATORY DISEASE USING CYCLOPHILIN RECEPTOR ANTAGONISTS

5  **TECHNICAL FIELD OF THE INVENTION**

The present invention provides a method for the treatment of HIV infection, acquired immune deficiency syndrome (AIDS), rheumatoid arthritis (RA), connective tissue disorders and cancer utilizing antagonists of the ligand/receptor relationship between cyclophilin (CyP) and the human extracellular matrix metalloproteinase inducer protein (EMMPRIN). The present

10  invention further provides anti-EMMPRIN antibodies and soluble forms of EMMPRIN or EMMPRIN peptides that provide therapeutic activity by modifying the CyP/EMMPRIN binding interaction. The present invention further provides screening assays for the identification of compounds that modify the CyP/EMMPRIN binding interaction.

15  **BACKGROUND OF THE INVENTION**

The present invention relates to cyclophilins, to their cognate interaction with cellular receptors for cyclophilins, and to the treatment of cyclolphilin-related disorders. For example, cyclophilin A is, *inter alia*, involved in the etiology of **(1)** the symptoms associated with acquired immune deficiency syndrome (AIDS) from HIV infection, and **(2)** inflammatory

20  disease (*e.g.*, rheumatoid arthritis).

**(1) Acquired Immune Deficiency Syndrome (AIDS)**

The human immunodeficiency virus (HIV) is a member of the lentivirus family of retroviruses (Teich et al., RNA Tumor Viruses, Weiss et al., eds. CSH-Press, pp.949-956, 1984). HIV has been implicated as the primary cause of the slowly degenerative immune system

25  disease termed acquired immune deficiency syndrome (AIDS) (Barre-Sinoussi et al., *Science* 220:868-870, 1983; Gallo et al., *Science* 224:500-503, 1984). In humans, HIV replication occurs prominently in CD4$^+$ T lymphocyte and macrophage populations, and HIV infection leads to depletion of T cells and eventually to immune incompetence, opportunistic infections, neurological dysfunctions, neoplastic growth, and ultimately death.

30  The core of the HIV viral particle comprises capsid antigen (CA) proteins, together with the viral RNA genome and those enzymes required for early replicative events. Myristylated matrix antigen (MA) protein fills the space between the viral core and a lipid membrane envelope derived from the infected cell membrane. The HIV envelope surface glycoproteins are synthesized as a single 160 Kd precursor protein that is cleaved by a cellular protease during

35  viral budding into two glycoproteins, gp41 and gp120. Viral gp41 is a transmembrane glycoprotein and gp120 is an extracellular glycoprotein that remains non-covalently associated with gp41, possibly in a trimeric or multimeric form (Hammarskjold & Redosh, *Biochem. Biophys. Acta* 989:269-280, 1989).

HIV is targeted to CD4$^+$ host cells because CD4 acts as the cellular receptor for the HIV-1 virus (Dalgleish et al., *Nature* 312:763-767, 1984; Klatzmann et al., *Nature* 312:767-768, 1984; Maddon et al., *Cell* 47:333-348, 1986). Viral entry into cells is dependent upon gp120 binding the cellular CD4 receptor molecules (McDougal et al., *Science* 231:382-385, 1986; Maddon et al., *Cell* 47:333-348, 1986), explaining HIV's tropism for CD4$^+$ cells, while gp41 anchors the envelope glycoprotein complex in the viral membrane. While these virus:cell interactions are necessary for infection, there is evidence that additional virus:cell interactions are also required.

HIV infection is pandemic and HIV-associated diseases represent a major world health problem. Unfortunately, no ultimately curative anti-retroviral drugs or vaccines against HIV infection and/or AIDS exist. Attempts to develop such drugs and vaccines have targeted molecules corresponding to various stages of the HIV life cycle (*e.g.*, reverse transcriptase, late-stage viral proteases, and in the case of vaccines gp160, gp120 and gp41). Attempts are also being made to develop drugs that can inhibit viral entry into the cell, the earliest stage of HIV infection. Here, the focus of therapeutic intervention has been on CD4 and CCR5, recognized cell-surface receptors for HIV. Recombinant soluble CD4, for example, has been shown to inhibit infection of CD4$^+$ T cells by some HIV-1 strains (Smith et al., *Science* 238:1704-1707, 1987). Certain primary HIV-1 isolates, however, are relatively less sensitive to inhibition by recombinant CD4 (Daar et al., *Proc. Natl. Acad. Sci. USA* 87:6574-6579, 1990). Moreover, recombinant soluble CD4 clinical trials have produced inconclusive results (Schooley et al., *Ann. Int. Med.* 112:247-253, 1990; Kahn et al., *Ann. Int. Med.* 112:254-261, 1990; Yarchoan et al., *Proc. Vth Int. Conf. on AIDS*, p. 564, MCP 137, 1989).

Therefore, although a great deal of effort is being directed to the design and testing of anti-retroviral drugs and vaccines, there is a need in the art for effective, non-toxic treatments.

*New Therapeutic Intervention Targets for AIDS; Cyclophilin A.* Cyclophilin A (hereinafter "CyPA") is a 19 KD protein that is expressed in a wide variety of cells. CyPA binds the immunosuppressive agent cyclosporin A (hereinafter "CsA") (Etzkorn et al., *Current Biology* 3:929-933, 1993; Liu, *Immunol. Today.* 14:290-295, 1993 [published erratum appears in *Immunol. Today* 14(8):399, 1993]), and possesses a peptidyl-prolyl cis-trans isomerase (PPIase) activity, and protein folding or "chaperone" activities (Galat, *Eur. J. Biochem.* 216:689-707, 1993; Kofron et al., *Biochemistry.* 30:6127-6134, 1991 [published erratum appears in *Biochemistry* 30(44):10818, 1991]). CyPA is a member of the "immunophilin family," that is a group of related cellular factors involved in regulating immunity (Galat, *Eur. J. Biochem.* 216:689-707, 1993; Fruman et al., *FASEB. J.* 8:391-400, 1994). At least four types of mammalian cyclophilins have been cloned, CyPA, CyPB, CyPC and hCyP3 (Friedman et al., *Proc. Natl. Acad. Sci. USA*, 90:6815-6819, 1993).

CyPA is recognized to be one of the host cell receptors for CsA, a potent immunosuppressive drug that is widely used in prevention of graft rejection. CsA is a member

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of a family of hydrophobic cyclic undecapeptides that exhibits potent immunosuppressive, antiparasitic, fungicidal and chronic anti-inflammatory properties. CsA is thought to exert its immunosuppressive effects by inhibiting the early stages in T cell activation. CsA has been found to block RNA transcription of the T cell growth factor interleukin 2 (IL-2), and to inhibit

5    expression of the IL-2 receptor by precursor cytolytic T lymphocytes (Palacios, *J. Immunol.* 128:337, 1982). CyPA binds to CsA with a dissociation constant in the range of $10^{-8}$ mol/L, a value consistent with levels needed to elicit immunosuppression (Handschumacher et al., *Science* 226:544, 1984).

A functional association of CyPA with the Gag protein of HIV virions, and specifically
10   with the capsid antigen portion thereof, has been identified (Luban et. al., *Cell*, 1993). CyPA is specifically incorporated into HIV-1 virions, and disruption of the Gag-cyclophilin interaction has been reported to prevent both incorporation of CyPA into virions, and HIV-1 replication (Franke et al., *Nature* 372:359-362, 1995; Thali et al., *Nature* 372:363-365, 1994). These data are consistent with a model whereby the interaction of Gag with CyPA is necessary for the
15   formation of infectious HIV-1 virions (Braaten et. al., *J.Virol.* 70:3551-3561, 1966).

CsA has been shown to interfere with Gag-cyclophilin interactions *in vitro*, block cyclophilin incorporation into virions, and inhibit the replication of HIV-1 in cell culture (Franke et al., *supra*; Thali et al., *supra*; Billich et al., *J. Virol.* 69:2451-2461, 1995). CsA has not been shown to interact directly with the HIV-1 virus. It is hypothesized that CsA, and its analogs,
20   interfere at two stages in the HIV life cycle. First, by interacting with CyPA during establishment of infection where CsA treatment inhibits HIV infection prior to integration into the genome of the infected cell as measured by formation of circular HIV DNA and integration of proviral DNA into the host genome (Sherry et al., *Proc. Natl. Acad. Sci. U.S.A.* 95:1758-1763, 1998). Second, by interacting with CyPA at a late stage of virus replication by preventing CyPA
25   incorporation into HIV-1 particles, thus rendering the virus non-infectious (Billich et al., *supra*). Therefore, research activity has focused on disrupting the interaction between the viral Gag protein, and the cellular CyPA protein in attempts to develop an anti-HIV agent.

Despite the foregoing observations, the usefulness of CsA as a treatment for HIV-infection in patients is severely limited. First, CsA is a potent immunosuppressive agent. Thus,
30   its use in HIV-infected patients, who will become immunocompromised, is contraindicated. Moreover, the HIV-inhibitory effects of CsA and its analogs require drug concentrations that are 10- to 100-fold higher than those necessary to effectuate immunosuppression with CsA. Somewhat paradoxically, the ability of cyclosporin to generally suppress the systemic immune system has been proposed as a treatment for HIV infection (*see* U.S. Patent No. 4,814,323).
35   However, this method of treatment has severe drawbacks, given the severely immunosuppressed state of HIV-infected patients.

Therefore, there is a need in the art to identify and characterize novel CyPA-related intervention targets and binding agents, and to use them to identify novel compounds and

methods for treating and preventing HIV infection. There is a need in the art to identify and mechanistically understand the means by which CyPA specifically interacts with cells to mediate HIV infection, and how such mechanisms can be used therapeutically, or to find novel therapeutic agents, or to provide novel and useful diagnostic and prognostic assays.

5       **(2) Rheumatoid Arthritis**

Rheumatoid arthritis (hereinafter "RA") is a chronic inflammatory disease of the joints. RA is associated with considerable morbidity, functional disability and increased mortality (Harris, *N. Engl. J. Med.* 322:1277-1289, 1990 [published erratum appears in *N. Engl. J. Med.* 323:996, 1990; see comments]; Brooks, *Lancet* 341:286-290, 1993). There are an estimated 2-4

10      million cases of RA in the U.S., with the peak incidence between ages 35 and 45 (Brooks, *supra*). The etiology of RA is unknown.

A persistent inflammatory synovitis, thought to be initiated by T cell-dependent mechanisms, is one of the hallmarks of RA, and usually affects the peripheral joints as a symmetric polyarthritis (Brooks, *supra*). This inflammatory synovitis is believed to be

15      exacerbated by (a) infiltrating neutrophils that release oxygen radicals and proteolytic enzymes, and (b) resident fibroblast-like synovial cells which release proenzyme forms of matrix metalloproteinases which are themselves activated by neutrophil proteases and subsequently cause cartilage degradation. Accordingly, synovial inflammation leads to cartilage destruction and bone erosion with subsequent joint deformities (Zvaifler & Firestein, *Arthritis Rheum.*

20      37:783-789, 1994; Sewell & Trentham, *Lancet* 341:283-286, 1993).

*T Cell Involvement.* Strong evidence for the role of T cells in the initiation and perpetuation of RA is accumulating, although other cell types (*e.g.*, dendritic cells, fibroblast-like synoviocytes, and primary B cells) contribute to the characteristic progressive joint destruction (Thomas et al., *J. Leukoc. Biol.* 66:286-292, 1999; McCachren, *Arthritis Rheum.*

25      34:1085-1093, 1991; Zvaifler & Firestein, *supra*). Considerable evidence implicates neutrophils, and neutrophil products in joint destruction (Edwards & Hallett, *Immunol. Today* 18:320-324, 1997; Harris, *supra*; Kakimoto et al., *Cell Immunol.* 165:26-32, 1995; Janusz & Durham, *Inflamm. Res.* 46:503-508, 1997; Griffiths et al., *Proc. Natl. Acad. Sci. U.S.A.* 92:517-521, 1995).

30      Thus, rheumatoid arthritis is a complex, progressive disease, involving multiple cell types. Therapeutic intervention strategies in RA patients have been primarily directed at blocking the onset and propagation of the disease, and secondarily at blocking local joint inflammation that mediates joint damage and injury (Harris, *N. Engl. J. Med.* 322:1277-1289, 1990 [published erratum appears in *N. Engl. J. Med.* 323:996, 1990; see comments]; Brooks,

35      *Lancet* 341:286-290, 1993).

The stimulus for synovial hyperplasia in RA has been classically described as an immune-mediated inflammatory process (Zvaifler & Firestein, *Arthritis Rheum.* 37:783-7899, 1994). Such inflammation is believed to be the response to factors made by activated CD4$^+$ T

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cells, the predominant cell in the subintimal infiltrates of the RA synovium (Zvaifler & Firestein, *Arthritis Rheum.* 37:783-7899, 1994; McCachren, *Arthritis Rheum.* 34:1085-1093, 1991). Moreover, the observation that specific class II MHC polymorphisms confer a risk for RA strongly suggests that T cells play a pivotal role in the development of RA (Gregersen et al., *Am.*
5   *J. Med.* 85:17-19, 1988; Winchester et al., *Rheum. Dis. Clin. North Am.* 18:761-783, 1992).

The revelation that T cells play an integral part in the pathogenesis of RA led to the use of cyclosporin A (hereinafter "CsA") as a therapeutic agent in patients with RA (Yocum, *Semin. Arthritis Rheum.* 29:27-35, 1999; Brooks, *Lancet* 341:286-290, 1993; de Wynter et al., *Stem.Cells* 16:349-356, 1998). CsA is a potent immunosuppressive agent presumed to act via
10   binding to intracellular stores of cyclophilin (Erlanger, *Immunol. Today.* 13:487-490, 1992; Liu, *Immunol. Today* 14:290-295, 1993 [published erratum appears in *Immunol. Today* 14(8):399, 1993]; Flanagan et al, *Nature.* 352:803-807, 1991 [see comments]).

*Non T Cell Factors in RA; Neutrophils and Matrix Metalloproteinases (MMPs).* Two arguments favor the crucial involvement of other factors, in addition to T cells, in RA-associated
15   pathology. Firstly, T cell products have not been demonstrated in RA synovial fluids or extracts (Edwards & Hallett, *Immunol. Today* 18:320-324, 1997). Secondly, current strategies based upon modifying T cell-mediated immune responses have not been efficacious, and can be accompanied by serious toxicity. Moreover, there is no evidence that such T cell-based strategies can limit the progression of bone and cartilage destruction, especially once the disease
20   has become established (*i.e.,* after about the first year) (Zvaifler & Firestein, *Arthritis Rheum.* 37:783-789, 1994; Yocum, *Semin. Arthritis Rheum.* 29:27-35, 1999).

*Neutrophils.* RA is characterized by the accumulation of a large number of neutrophils in the synovial fluid. Chemo attractants, such as C5a, leukotriene B$_4$, platelet activating factor (PAF), and IL-8 (Harris, *N. Engl. J. Med.* 322:1277-1289, 1990 [published erratum appears in *N.*
25   *Engl. J. Med.* 323:996, 1990; see comments]; Edwards & Hallett, *Immunol. Today* 18:320-324, 1997; Premack & Schall, *Nature Med.* 2:1174-1178, 1996), are believed to play a role in eliciting the characteristic neutrophil influx. Neutrophils are activated within the joint fluid, most likely by the phagocytosis of cellular debris and immune complexes, resulting in degranulation and release of proteinases and reactive oxidants (Harris, *N. Engl. J. Med.*
30   322:1277-1289, 1990 [published erratum appears in *N. Engl. J. Med.* 323:996, 1990; see comments]). Additionally, activated neutrophils secrete many of the cytokines that are detectable in RA synovial fluid: IL-1β, IL-8, IL-12, TNF-α, TGF-, and GRO-α (Edwards & Hallett, *Immunol. Today* 18:320-324, 1997). Neutrophils also play a central role in tissue destruction, most likely because they release enzymes (*e.g.,* elastase), into the joint where they
35   are destructive (Sewell & Trentham, *Lancet* 341:283-286, 1993; Edwards & Hallett, *Immunol. Today* 18:320-324, 1997). Accordingly, blocking neutrophil elastase with specific inhibitors (*e.g.,* MDL 101, 146 and ONO-5046) results in decreases in cartilage destruction in animal

models of arthritis (Janusz & Durham, *Inflamm.Res.* 46:503-508, 1997; Kakimoto et al., *Cell Immunol.* 165:26-32, 1995).

*Matrix Metalloproteinases (MMPs).* Matrix metalloproteinases (hereinafter "MMPs") comprise a group of structurally related zinc metalloendopeptidases capable of degrading
5    extracellular matrix components (Nagase et al., *Matrix Suppl.* 1:237-244, 1992; Giambernardi et al., *Matrix Biol.* 16:483-496, 1998). MMPs play an important beneficial role in normal tissue remodeling processes, wherein MMP activities are precisely controlled in various cell types. MMP activity is not only controlled at the level of cellular gene expression, but also at the protein level, either by precursor activation (proMMP to MMP) or inhibition of activated species
10   by endogenous inhibitors, *e.g.*, by $\alpha_2$-macroglobulin and tissue inhibitors of metalloproteinases (TIMPs) (Nagase et al., *Matrix Suppl.* 1:237-244, 1992; Ogata et al., *J. Biol. Chem.* 270:18506-18511, 1995; Zucker et al., *J. Biol. Chem.* 273:1216-1222, 1998).

However, the deregulated action of MMPs contributes to the pathological destruction of the extracellular matrix in many connective tissue diseases such as arthritis, periodontitis, tissue
15   ulceration, and in cancer cell invasion and metastasis (Kahariet al., *Exp. Dermatol.* 6:199-213, 1997; Keyszer et al., *J. Rheumatol.* 26:251-258, 1999; Benbow et al., *J. Biol. Chem.* 274:25371-25378, 1999; Keyszer et al., *Z. Rheumatology* 57:392-398, 1998).

*New Therapeutic Intervention Targets for RA: Cyclophilins.* The exact cause of pathologic processes in RA remains controversial. Traditionally, treatment of RA has involved a
20   multidisciplinary approach with combinations of non-steroidal anti-inflammatory drugs (NSAIDs) and slow-acting anti-rheumatic drugs (SAARDs). Such combination therapies are often effective, but result in significant side effects. For example, NSAIDs provide significant symptomatic relief of joint inflammation, but can accumulate in kidney and gut leading to adverse reactions, including gastrointestinal distress, renal damage, and skin reactions (Yocum,
25   *Semin. Arthritis Rheum.* 29:27-35, 1999; Brooks, *Lancet* 341:286-290, 1993).

Therefore, there is a need in the art to identify new therapeutic targets that would allow for the design of new treatment strategies for RA. Three broad pathological-process areas for therapeutic intervention in RA are: aberrant T cell activation responses; abnormal proliferation of, and excessive cytokine and MMP production by synovial cells; and leukocyte migration from
30   the peripheral blood to extravascular sites.

*Cyclophilin A.* One specific therapeutic target, relevant to all three of the above-mentioned process areas, is cyclophilin A (hereinafter "CyPA") the ubiquitously-distributed host cell receptor for CsA. CyPA is not entirely an intracellular protein, but is also secreted by cells in response to inflammatory stimuli (Sherry et al., *Proc. Natl. Acad. Sci. USA* 89:3511-3515,
35   1992). Extracellular CyPA is a potent neutrophil and eosinophil chemoattractant *in vitro*, and elicits an inflammatory response characterized by a rapid influx of neutrophils when injected *in vivo* (Sherry et al., *Proc. Natl. Acad. Sci.USA* 89:3511-3515, 1992; Xu.et al., *J. Biol. Chem.* 267:11968-11971, 1992; Leiva & Lyttle, *Biochem. Biophys. Res. Commun.* 186:1178-11836,

1992). The leukocyte attractant activity of CyPA is blocked in the presence of CsA, but not by cyclosporin G, a non-immunosuppressive analogue of CsA (Sherry et al., *Proc. Natl. Acad. Sci.USA* 89:3511-3515, 1992; Xu et al., *J. Biol. Chem.* 267:11968-11971, 1992).

5     The synovial fluid in the joints of RA patients contains high levels of CyPA, and that such CyPA levels correlate both with the number of neutrophils in the synovial fluid and with disease severity (Billich et al., *J. Exp. Med.* 185:975-980, 1997). It is logical to presume that the CyPA released into the synovial fluid is attracting neutrophils, which become activated upon arrival and, in turn, release proteolytic enzymes and oxidants that promote cartilage destruction and exacerbate disease. Thus, the effectiveness of CsA in alleviating RA may be manifested in

10 two ways. Firstly, it may act via its ability to interfere with T cell activation and therefore the initiation of the disease process. Secondly, CsA may act by binding to the CyPA released in the joints during periods of active disease, and thereby blocking CyPA's neutrophil chemo-attractant activity.

    However, CsA treatment is inherently toxic, resulting in liver destruction, immune

15 suppression and susceptibility to opportunistic infections. These toxic effects are the result of the fact that CsA is membrane permeable and readily enters into all cells.
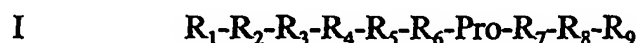
    Therefore, there is a need in the art to gain additional understanding of the basic pathophysiology of RA etiology and progression, of HIV infection, and of exacerbating mechanisms for these processes. Accordingly, there is a need in the art to identify and

20 characterize novel cyclophilin-related intervention targets, and to use such targets to identify novel compounds and methods for treating inflammatory disease and preventing HIV infection. There is a need in the art to identify and mechanistically understand the means by which cyclophilins specifically interact with cells to mediate signal transduction and HIV infection. Finally, there is a need in the art to understand how such mechanisms, once identified, can be

25 used therapeutically, or to find novel therapeutic agents, or to provide novel and useful diagnostic and prognostic assays these these conditions.

## SUMMARY OF THE INVENTION

    The present invention is based upon the surprising discovery that the previously known

30 extracellular matrix metalloproteinase inducer protein ("EMMPRIN") [SEQ ID NO:2], also known as CD147, is a novel cell-surface receptor for cyclophilins ("CyP"). Accordingly, the examples and embodiments of the present invention provide for *methods of treatment*, *pharmaceutical compositions*, and for *screening assays* for test compounds having therapeutic activity for indications such as HIV-1 infection, acquired immune deficiency syndrome

35 ("AIDS"), inflammatory and autoimmune disease (*e.g.*, rheumatoid arthritis ("RA"), EAE, ARDS and peridontitis), connective tissue disorders, tumor growth or metastasis or any condition characterized by local or systemic CyP release, synthesis or binding in a patient needing treatment.

*Methods of Treatment.* The present invention provides a method for treating or preventing inflammatory disease, autoimmune disease, tissue ulceration, tumor growth or metastasis or any condition characterized by local or systemic CyP release, synthesis or binding in a patient needing treatment, comprising administering to the patient a therapeutically effective

5      amount of a CyP/EMMPRIN antagonist or agonist. Preferably, the CyP/EMMPRIN antagonist or agonist is an anti-EMMPRIN antibody, a soluble EMMPRIN protein or polypeptide, a CyP protein or polypeptide and combinations thereof. Preferably, the inflammatory or autoimmune disease is arthritis, EAE, ARDS or peridontitis. Preferably the CyP/EMMPRIN antagonist or agonist is an anti-EMMPRIN antibody. Preferably, the anti-EMMPRIN antibody binds to the

10     extracellular domain or the transmembrane domain of EMMPRIN. Preferably, the anti-EMMPRIN antibody is a monoclonal antibody. Preferably, the anti-EMMPRIN monoclonal antibody is a single-chain antibody, chimeric antibody, humanized antibody, or Fab fragment. Most preferably, the anti-EMMPRIN antibody binds to an epitope that is recognized by the UM-8D6 anti-CD147 monoclonal antibody. Preferably, the CyP/EMMPRIN antagonist or agonist is

15     an EMMPRN polypeptide comprising an amino acid sequence of about 5 to 14 amino acids taken from a sequence extending from about residue 206 to about residue 229 of [SEQ ID NO:2] corresponding to the EMMPRIN transmembrane domain.

The present invention further provides a method for treating or preventing inflammatory disease, autoimmune disease, tissue ulceration, tumor growth or metastasis or any condition

20     characterized by local or systemic CyP release, synthesis or binding in a patient needing treatment, comprising administering to the patient a therapeutically effective amount of a compound of forumla I wherein formula I is

$$I \qquad R_1\text{-}R_2\text{-}R_3\text{-}R_4\text{-}R_5\text{-}R_6\text{-}Pro\text{-}R_7\text{-}R_8\text{-}R_9$$

25

wherein: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$, $R_3$ and $R_4$ are independently Leu, Ile, Ala, Met or nothing; $R_5$ and $R_8$ are independently Leu, Ile, Ala or Met; $R_6$ and $R_7$ are independently Phe, Trp or Tyr; and -$R_9$ is -Gly, -GlyIle, -GlyIleVal or nothing. Preferably, for formula I: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$ is

30     Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$ is Phe; $R_8$ is Leu; and -$R_9$ is nothing. Preferably, for formula I: $R_1$- is nothing; $R_2$ is Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$ is Phe; $R_8$ is Leu; and -$R_9$ is nothing.

The present invention further provides a method for decreasing the susceptibility of a cell to retroviral infection, wherein the cell expresses a functional EMMPRIN receptor, and wherein

35     a retrovirus uses the EMMPRIN receptor in a pre-integration step of viral infection, comprising contacting the cell with an amount of a CyP/EMMPRIN antagonist or agonist sufficient to inhibit the pre-integration step of retroviral infection. Preferably, the CyP/EMMPRIN antagonist or agonist is an anti-EMMPRIN antibody, a soluble EMMPRIN protein or peptide, a

CyP derivative or peptide, and combinations thereof. Preferably the CyP/EMMPRIN antagonist or agonist is an anti-EMMPRIN antibody. Preferably, the anti-EMMPRIN antibody binds to the extracellular domain or the transmembrane domain of EMMPRIN. Preferably, the anti-EMMPRIN antibody is a monoclonal antibody. Preferably, the anti-EMMPRIN monoclonal

5      antibody is a single-chain antibody, chimeric antibody, humanized antibody, or Fab fragment. Most preferably, the anti-EMMPRIN antibody binds to an epitope that is recognized by the UM-8D6 anti-CD147 monoclonal antibody. Preferably, the CyP/EMMPRIN antagonist or agonist is an EMMPRN polypeptide comprising an amino acid sequence of about 5 to 14 amino acids taken from a sequence extending from about residue 206 to about residue 229 of [SEQ ID NO:2]

10     corresponding to the EMMPRIN transmembrane domain.

The present invention further provides a method for decreasing the susceptibility of a cell to retroviral infection, wherein the cell expresses a functional EMMPRIN receptor, and wherein a retrovirus uses the EMMPRIN receptor in a pre-integration step of viral infection, comprising contacting the cell with an amount of a compound of formula I sufficient to inhibit the pre-

15     integration step of retroviral infection, wherein formula I is

$$I \qquad R_1\text{-}R_2\text{-}R_3\text{-}R_4\text{-}R_5\text{-}R_6\text{-}Pro\text{-}R_7\text{-}R_8\text{-}R_9$$

wherein: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$, $R_3$ and $R_4$ are

20     independently Leu, Ile, Ala, Met or nothing; $R_5$ and $R_8$ are independently Leu, Ile, Ala or Met; $R_6$ and $R_7$ are independently Phe, Trp or Tyr; and -$R_9$ is -Gly, -GlyIle, -GlyIleVal or nothing. Preferably, for formula I: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$ is Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$ is Phe; $R_8$ is Leu; and -$R_9$ is nothing. Preferably, for formula I: $R_1$- is nothing; $R_2$ is Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$

25     is Phe; $R_8$ is Leu; and -$R_9$ is nothing.

The present invention further provides a method for treating or preventing HIV infection, AIDS or AIDS-related disorders in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of a CyP/EMMPRIN antagonist or agonist. Preferably, the CyP/EMMPRIN antagonist or agonist is an anti-EMMPRIN antibody, a soluble

30     EMMPRIN protein or peptide, a CyP derivative or peptide, and combinations thereof. Preferably the CyP/EMMPRIN antagonist or agonist is an anti-EMMPRIN antibody. Preferably, the anti-EMMPRIN antibody binds to the extracellular domain or the transmembrane domain of EMMPRIN. Preferably, the anti-EMMPRIN antibody is a monoclonal antibody. Preferably, the anti-EMMPRIN monoclonal antibody is a single-chain antibody, chimeric

35     antibody, humanized antibody, or Fab fragment. Most preferably, the anti-EMMPRIN antibody binds to an epitope that is recognized by the UM-8D6 anti-CD147 monoclonal antibody.

The present invention further provides a method for treating or preventing HIV infection, AIDS or AIDS-related disorders in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a compound of formula I, wherein formula I is

5          I                    $R_1$-$R_2$-$R_3$-$R_4$-$R_5$-$R_6$-Pro-$R_7$-$R_8$-$R_9$

wherein: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$, $R_3$ and $R_4$ are independently Leu, Ile, Ala, Met or nothing; $R_5$ and $R_8$ are independently Leu, Ile, Ala or Met; $R_6$ and $R_7$ are independently Phe, Trp or Tyr; and -$R_9$ is -Gly, -GlyIle, -GlyIleVal or nothing.

10    Preferably, for formula I: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$ is Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$ is Phe; $R_8$ is Leu; and -$R_9$ is nothing. Preferably, for formula I: $R_1$- is nothing; $R_2$ is Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$ is Phe; $R_8$ is Leu; and -$R_9$ is nothing.

*Screening assays.* The present invention further provides a method for identifying test
15   compounds having therapeutic activity for HIV infection, AIDS or AIDS-related disorders, inflammatory disease, autoimmune disease, tumor growth or metastasis or any condition characterized by local or systemic CyP release, synthesis or binding in a patient needing treatment, comprising: (a) contacting a test compound with a functional CyP protein and a functional EMMPRIN protein wherein at least one of the proteins bears a detectable label; (b)
20   assaying any resulting EMMPRIN/CyP complex for the presence of the label; and (c) determining whether the test compound inhibited binding of the CyP protein to the EMMPRIN protein, whereby test compounds that inhibit binding of the CyP protein to the EMMPRIN protein are identified as therapeutic compounds. Preferably, either the functional EMMPRIN protein or the functional CyP protein is immobilized onto a solid phase. Preferably, the CyP
25   protein or the EMMPRIN protein is labeled with a radiolabel, a fluorescent reporter or quencher moiety, an enzymic label that catalyzes a colorometric or fluorometric change or combinations thereof. Preferably, the inflammatory or autoimmune disease is arthritis, EAE, ARDS or peridontitis.

The present invention further provides a method for identifying test compounds having
30   therapeutic activity for HIV infection, AIDS or AIDS-related disorders, inflammatory disease, autoimmune disease, tumor growth or metastasis or any condition characterized by local or systemic CyP release, synthesis or binding in a patient needing treatment, comprising: (a) contacting a test compound, in the presence of a functional CyP protein, with a cell expressing a functional EMMPRIN protein; and (b) determining whether the test compound inhibits binding
35   of the CyP protein to the EMMPRIN protein, whereby test compounds that inhibit binding of the CyP protein to the EMMPRIN protein are identified as therapeutic compounds. Preferably, the inflammatory or autoimmune disease is arthritis, EAE, ARDS or peridontitis. Preferably, the cell expresses recombinant EMMPRIN, CD4, CXCR4 or combinations thereof. Preferably,

10

determination of the inhibition of binding of the CyP protein to the EMMPRIN protein is based on an assay selected from the group consisting of CyP/EMMPRIN antagonist assays, receptor sensitization or desensitization assays, receptor up- or down-regulation assays, EMMPRIN-mediated signal transduction assays, $Ca^{2+}$ mobilization assays, matrix metalloproteinase

5    expression or activity assays, cell growth rate assays, HIV-1 infection assays, and assays based on detection of a specific marker of cell cycle or cell differentiation. Preferably, the EMMPRIN-mediated signal transduction assays are based on determining the phosphorylation or activation status of an intracellular protein, wherein the intracellular protein is selected from the group consisting of Ras, PKA, RAP1, B-Raf, Mek, and MAPK, whereby test compounds that alter the

10   phosphorylation or activation status of said intracellular proteins, relative to their phosphorylation or activation status in control cells, are identified as therapeutic compounds. Preferably, the specific marker of cell cycle or cell differentiation is selected from the group consisting of cell-cycle regulatory proteins cyclin D1, cyclin E and p21/Waf1, whereby test compounds that alter said cell-cycle markers, relative to their status in control cells, are

15   identified as cancer therapeutic compounds. Preferably, the matrix metalloproteinase expression assays measure the expression or activity of proMMP-1, proMMP-3, proMMP-9, MMP-1, MMP-3 or MMP-9. Preferably, the HIV-1 infection assays measure viral reverse transcriptase activity or virus-directed luciferase expression.

*Pharmaceutical compositions*. The present invention further provides a pharmaceutical
20   composition comprising a compound of formula I or a pharmaceutically-acceptable salt thereof, and a pharmaceutically-acceptable carrier, wherein formula I is

I            $R_1$-$R_2$-$R_3$-$R_4$-$R_5$-$R_6$-Pro-$R_7$-$R_8$-$R_9$

25   wherein: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$, $R_3$ and $R_4$ are independently Leu, Ile, Ala, Met or nothing; $R_5$ and $R_8$ are independently Leu, Ile, Ala or Met; $R_6$ and $R_7$ are independently Phe, Trp or Tyr; and -$R_9$ is -Gly, -GlyIle, -GlyIleVal or nothing. Preferably, for formula I: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$ is Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$ is Phe; $R_8$ is Leu; and -$R_9$ is nothing.

30   Preferably, for formula I: $R_1$- is nothing; $R_2$ is Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$ is Phe; $R_8$ is Leu; and -$R_9$ is nothing.


**BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 (left-hand panel) shows a schematic representation of the human EMMPRIN
35   protein encoded by the cDNA clone isolated according to the present invention [SEQ ID NO:1]. The 269-amino acid EMMPRIN protein is divided into several functional domains, including signal peptide (sp), extracellular domain (ecd), transmembrane domain (tm), and intracellular domain (icd). Both EMM.C1 and EMM.C2 constructs used for binding studies lacked the signal

peptide, and one (EMM.C1) had only the extracellular domain. The right right-hand panel of
Figure 1 shows that [$^{35}$S]-labeled EMMPRIN was specifically retained by CyPA-sepharose
affinity resin. The affinity-bound EMMPRIN was eluted and analyzed by electrophoresis and
autoradiography. No EMMPRIN was retained by the control resin lacking CyPA.

5        Figure 2 shows a FACS analysis of EMMPRIN expression in CHO cells stably
transfected with empty vector (CHO), or in an EMMPRIN-expressing CHO cell line (CHO-
EMM) EMMPRIN expression was analyzed by FACS using anti-CD147 (M6FT), a
commercially-available anti-EMMPRIN antibody (Research Diagnostics, Inc.; Flanders, NJ).
CHO cells can be stably transfected to express high levels of EMMPRIN.

10       Figure 3 shows that Ca$^{2+}$ mobilization in CHO (no EMMPRIN expression) or CHO-
EMM cells (stable constitutive EMMPRIN expression) was induced by exogenously-added
CyPA (10 μg/ml) (left-hand panel). CyPA-induced signaling in CHO-EMM cells was blocked
by anti-CD147 mAb (M6FT) antibody (i.e., anti-EMPRIN mAB), but not by isotype control
antibody (right-hand panel).

15       Figure 4 shows that genistein (a tyrosine kinase inhibitor) completely abrogated CyPA-
induced, EMMPRIN-mediated Ca$^{2+}$ mobilization in CHO-EMM cells. CyPA-induced Ca$^{2+}$
mobilization was measured in CHO-EMM cells pretreated with various inhibitors of various
kinases known to be involved in the transduction of intracellular signals. The curves show, from
upper to lower, pretreatment with no inhibitor, pertussis toxin (PTX) B-oligomer (induces cross-

20       desensitization of chemokine receptors), PTX (inactivates signaling pathways mediated by
members of the Gi-Go and Gt-protein family), PD98059 (a MAPK inhibitor),
bisindolylmaleimide I (a PKC inhibitor ) and genistein. Genistein completely abrogated CyPA-
induced Ca$^{2+}$ mobilization in CHO-EMM cells.

         Figure 5 shows a Western immunoblot analysis of the effect of CyPA treatment on

25       matrix metalloproteinase (MMP) expression in WI-38 human primary lung fibroblasts. Human
WI-38 fibroblasts were treated with CyPA or media alone, and supernatant fluids were
subsequently collected, filtered, and analyzed by western immunoblots for MMP-1, MMP-3 and
MMP-9 reactivity using mouse monoclonal antibodies (Cal Biochem, La Jolla, CA). CyPA-
treatment activated expression and release of proMMP-1, and enhanced that of proMMP-3.

30       Figure 6 shows analyses of luciferase (left-hand and middle panels) and HIV-1 reverse
transcriptase activity (right-hand panel) demonstrating that cell-surface EMMPRIN expression
enhances MuLV- and HIV-1$^{LAV}$-pseudotyped HIV-1 (Luc-HIV-1) infection of CHO-EMMPRIN
(CHO-EMM) cells relative to CHO (control) cells, that the enhancing effect is greatly reduced
by anti-CD147 mAb (UM-8D6), and that the enhancing effect is manifested at a stage preceding

35       integration of the proviral DNA.

         The left-hand panel shows luciferase expression (measured on day 4 post-infection, and
expressed as a percentage relative to control) in CHO (control) or CHO-EMM (constitutively
expressing EMMPRIN) cells that were infected with luciferase-expressing MuLV-pseudotyped

HIV-1. The presence of cell-surface EMMPRIN increased luciferase expression 5- to 6-fold. Moreover, this increase was greatly-reduced when anti-CD147 mAb (UM-8D6) was added during infection.

   The middle panel shows luciferase expression in triplicate cultures of CHO or CHO-
5 EMM cells that were transiently transfected with CD4- and CXCR-expressing vectors and then infected with Luc-HIV-1, which is pseudotyped with Env derived from HIV-1$^{LAV}$ that depends on CD4 and CXCR4 for entry. Luciferase expression is presented as percentage of expression relative to control (CHO/CD4/CXCR4 cells; taken as 100%). A 3- to 4-fold increase in luciferase expression was observed in cells expressing EMMPRIN.

10   The right-hand panel shows reverse transcriptase activity in CHO and CHO-EMM cells that were transiently transfected with CD4- and CXCR4-expressing vectors, and then infected with either Env$^{MuLV}$- or Env$^{LAI}$-pseudotyped luc-HIV-1 in the presence of anti-CD147 mAb (UM-8D6) or isotype-matched irrelevant antibody. Viral DNA was analyzed by PCR 2 hr after infection using primers LTR R/U5 specific for the early reverse transcription products. Results
15 were quantified on a Direct Imager (Packard) and are presented as radioactivity associated with LTR/*gag*-specific signal relative to control (isotype-treated CHO/CD4/CXCR4 cells; taken as 100%). The amount of LTR R/U5 reverse transcription product following infection with Env$^{MuLV}$ and Env$^{LAV}$-pseudotyped viruses was increased 3- to 4-fold in CHO-EMM/CD4/CXCR4 cells compared to CHO/CD4/CXCR4 cells. Moreover, anti-CD147 mAb diminished the
20 enhancing effect of EMMPRIN.

   Figure 7A shows that anti-CD147 mAb inhibited HIV-1 replication in human primary PBMC. Triplicate cultures of PHA-activated PBMC were infected with either the T-lymphotropic X4 strain HIV-1$^{LAV}$ or the macrophage-tropic R5 HIV-1$^{ADA}$ (left-hand panel), or with luciferase-expressing recombinant HIV-1 pseudotyped with envelopes derived from LAV
25 or ADA strains (right-hand panel). Anti-CD147 mAb (UM-8D6) or isotype-matched control mAb were added 30 min. prior to infection and were present throughout the duration of the experiment. Virus replication was assessed by RT activity in culture supernatants on day 21 (left-hand panel), or by luciferase activity on day 4 after infection (right-hand panel). Results are presented as RT or luciferase activity relative to control (cells treated with isotype-matched
30 irrelevant mAb; taken as 100%). The results show that EMMPRIN was involved in HIV-1 infection.

   Figure 7B shows that anti-CD147 mAb did not block HIV attachment to human PBMC. Triplicate PBMC cultures were pre-treated at 37°C with anti-CD147 mAb (UM-8D6; at 50 μg/ml), anti-CD4 mAb (5 μg/ml), or heparinase III ($3 \times 10^{-4}$ IU/ml), and then washed and
35 incubated with HIV-1$^{LAV}$ (10 ng/ml of p24) at 4°C for 1 hr. The amount of cell-bound viral p24 was assayed by ELISA. The results shown are for one representative experiment out of three performed with PBMC from different donors. Anti-CD147 mAb (UM-8D6) did not block HIV-1$^{LAV}$ attachment to human PBMC, whereas anti-CD4 mAb significantly reduced attachment.

Figure 7C shows that anti-CD147 mAb neither blocked HIV Env-mediated fusion between PBMC and HeLa cells expressing HIV-1 ADA and LAV envelopes, nor reduced cellular internalization of viral p24. *Left panel.* Fusion between PBMC and HeLa cells expressing HIV-1 Env (X4 LAV or R5 Ba-L) was assessed in the presence of anti-CD147 (UM-

5 8D6; at 50 µg/ml) or anti-CD4 (5 µg/ml) mAb by β-Gal expression (*see* "Methods," Example 6). Results are expressed as β-Gal activity relative to positive control (untreated cells; taken as 100%), and are presented as mean ± SD of three independent measurements with cells from the same donor. *Right panel.* For analysis of virus-cell fusion, PBMC were incubated with HIV-1$^{LAV}$ (see "Methods," Example 6). Cells were then treated with trypsin (0.025% for 30 min. at

10 37°C), lysed, and viral p24 was measured in the lysates by ELISA. These data show that the inhibitory effect of anti-CD147 mAb (UM-8D6) on HIV-1 infection was mediated by a post-fusion mechanism.

Figure 7D shows that anti-CD147 mAb reduced the amount of HIV-1 reverse transcription. *Upper-left and right-hand panels.* PHA-activated human PBMC were treated

15 with either anti-CD147 mAb (UM-8D6; at 50 µg/ml), or anti-CD4 mAb (2 µg/ml), or with corresponding isotype-matched control mAb (50 µg/ml) 2 hr before infection with HIV-1. Analysis of HIV-1 reverse transcription was performed 2 hr after infection of the PBMCs with HIV-1$^{LAV}$, using LTR R/U5 primers specific for the "strong-stop" early reverse transcription products. The PCR results were quantified on a Direct Imager (Packard) and are presented as

20 percentage of counts in treated, relative to antibody-untreated control (taken as 100%). The anti-CD147 mAb (UM-8D6) reduced the amount of the reverse transcription product by approximately 2-fold. As expected (*see* Example 6), anti-CD4 mAb reduced the amount of LTR R/U5-amplified fragment even more dramatically. *Lower-left panel.* Dilutions of 8E5/LAI cells containing one HIV-1 genome per cell were used as PCR standards.

25 Figure 7E shows the effect of anti-CD147 mAb on subcellular distribution of HIV-1 proteins. MT-4 cells were inoculated at 4°C with HIV-1$_{LAI}$ in the presence of 50 µg/ml of anti-CD147 mAb (Ancell) or isotype-matched control mAb. An aliquot (1x10$^6$ cells) was withdrawn after 30 minutes for protein analysis (*i.e.*, the 0 hr p.i. time-point sample) (*upper panel*), while the inoculated cultures were transferred to 37°C and incubated for 1.5 hr (*lower panels*).

30 Subcellular fractionation was performed (*see* "Methods," Example 6) and proteins in cytosolic (C), membrane (M), and cytoskeleton (CS) fractions were analyzed by Western blot and ECL using monoclonal antibodies to actin, CA, and MA. Viral MA protein and CA, in the absence of anti-CD147 mAb, underwent characteristic translocations from the membrane (M), into the cytoskeleton (CS), and cytosol fractions, respectively, 1.5 hours after infection (isotype panel,

35 lanes "CS" and "C," respectively). However, both MA and CA remained associated with the cell-membrane fraction in MT-4 cells treated with anti-CD147 mAb (UM-8D6) (α-CD147 panel, lane "M"), indicating that EMMPRIN was involved in the regulation of HIV-1 uncoating.

Figure 8 shows that replication of a CsA-resistant HIV virus strain was not inhibited by anti-CD147 mAb. 293T cells were-transfected with molecular clones of either HIV-$1_{NL4-3}$ ("WT") (*left panel*) or mutant ("A224E") (*right panel*) viruses, in the absence ("C") or presence ("CsA") of cyclosporin A (1 μM). Virus-producing transfected cells were washed and cultured

5   in quadruplicate for additional 4 days with ("293T + $CD4^+$ T cells") or without ("293T") addition of $CD8^+$ T cell-depleted PBMC and anti-CD147 mAb (UM-8D6; at 100 μg/ml). Virus replication was assayed by measuring RT activity in culture supernatants. Results are presented as mean ± SE. Anti-CD147 mAb (UM-8D6) did not inhibit replication of the CsA-resistant A224E virus, whereas replication of the wild-type virus was significantly diminished.

10  Figure 9 shows that an eight-amino acid polypeptide ([SEQ ID NO:3]) from the amino-terminal end of the EMMPRIN transmembrane region blocked infection of CHO-EMM cells by $Env^{MuLV}$-pseudotyped luciferase-expressing HIV-1. The left-hand panel shows a schematic structure of CD147 ("sp"= signal peptide, "e"= extracellular domain, "t"= transmembrane domain and "c"= cytoplasmic domain) and two peptides ("peptide 1" and "peptide 2") derived

15  from its transmembrane domain. The right-hand panel shows luciferase expression data on day 4 post-infection of CHO and CHO-EMM cells that were infected with recombinant $Env^{MuLV}$-pseudotyped luciferase-expressing HIV-1 in the presence of the indicated concentrations of peptides 1, 2 and "scrambled peptide 1." Peptide 1 (LAALWPFL) is an eight-amino acid sequence [SEQ ID NO:3] corresponding to amino acids 206-213 of the EMMPRIN

20  transmembrane domain. Peptide 2 (GIVAEVLVL) is a nine-amino acid polypeptide sequence [SEQ ID NO:4] corresponding to amino acids 214-222 of the EMMPRIN transmembrane domain. Scrambled peptide 1 (FAWPLLLA), the randomized sequence control, was made according to a randomized sequence of the amino acid residues of peptide 1.

25  **DETAILED DESCRIPTION OF THE INVENTION**
**Definitions**

CyP refers to the protein cyclophilin of which at least four types are known, CyPA (the host-cell receptor for cyclosporin A), CyPB, CyPC and hCyP3, any derivative of cyclophilin, or any fragments or peptides having an amino acid sequence corresponding to cyclophilin.

30  Polypeptides or peptide fragments of CyP are referred to as CyP polypeptides or CyP peptides, and include derivatives of CyP, CyP polypeptides or peptide fragments, and fusions of CyP, CyP polypeptides or peptide fragments to an unrelated protein (referred to herein as CyP derivatives, and CyP fusion proteins, respectively). A functional CyP refers to a protein that binds to its cognate CyP-binding protein or ligand *in vivo* or *in vitro*. For example, a functional CyPA is a

35  CyPA protein, peptide, polypeptide or fusion protein that binds to EMMPRIN, CsA, HIV-gag protein (specifically, with the capsid antigen portion thereof), or combinations thereof.

CyP binding protein means any cellular binding protein, cell-surface binding protein or receptor, extracellular receptor, soluble receptor or intracellular binding protein, which has

cyclophilin-specific binding activity. For example, as disclosed herein, EMMPRIN is a cell-surface CyP-binding protein (*i.e.*, a cell-surface receptor for CyP).

CsA is cyclosporin A, any derivative of cyclosporin A, or any fragments or peptides having an amino acid sequence corresponding to cyclosporin A.

5      EMMPRIN [SEQ ID NO:2] is the human extracellular matrix metalloproteinase inducer protein, and is a cell-surface CyPA or CyPB receptor. EMMPRIN is alternately referred to in the art as CD147, basigin and M6-antigen. Polypeptides or peptide fragments of EMMPRIN are referred to as EMMPRIN polypeptides or EMMPRIN peptides, and include derivatives of EMMPRIN, EMMPRIN polypeptides or peptide fragments, and fusions of EMMPRIN,

10     EMMPRIN polypeptides or peptide fragments to an unrelated protein (referred to herein as EMMPRIN derivatives, and EMMPRIN fusion proteins, respectively). A functional EMMPRIN protein, peptide, polypeptide or fusion protein refers to an EMMPRIN protein, peptide, polypeptide, fusion protein, cellular receptor, soluble receptor or intracellular binding protein that binds CyP protein, peptides or derivatives thereof, or that binds retroviral-associated CyPA

15     proteins or peptides *in vivo* or *in vitro*.

CD147 means EMMPRIN [SEQ ID NO:2].

Anti-EMMPRIN antibody means an anti-CD147 antibody.

Anti-CD147 or anti-EMMPRIN antibody means an antibody that specifically recognizes one or more epitopes of EMMPRIN, or conserved variants of EMMPRIN or peptide fragments

20     of EMMPRIN, including but not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single-chain antibodies, Fab fragments, F(ab')$_2$ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above.

Anti-CD147 mAb (UM-8D6), or UM-8D6 anti-CD147 monoclonal antibody specifically

25     refers to the murine IgG1 anti-CD147 monoclonal antibody that is commercially available from Ancell Immunology Research Products (Bayport, MN). This antibody was used in Examples 5-7 below.

Anti-CD147 mAB (M6FT) specifically refers to the anti-CD147 monoclonal antibody RDI-CD147-M6FT, that is commercially available from, Research Diagnostic, Inc. ("RDI,"

30     Flanders, NJ). This antibody was used in Example 2 below.

MMPs means matrix metalloproteinases. ProMMP means the inactive zymogen (proenzyme) form of the corresponding MMP that can be activated by proteolytic cleavage. For example, MMP-1 is produced by proteolytic cleavage of proMMP-1.

PIC means HIV-1 pre-integration complex.

35     MA means the HIV-1 matrix antigen protein.

CA means the HIV-1 capsid antigen protein.

PHA means the phytohaemaglutinin antigen.

293T cells refer to the art-recognized, HIV-1-susceptible human embryonic kidney cell

line.

CHO cells refer to the art-recognized Chinese Hamster ovary cell line.

CHO-EMM cells refer to the stably-transfected CHO cell line that constitutively expresses high levels of EMMPRIN.

MT-4 cells refer to the art-recognized human T cell line.

PBMC refers to primary human peripheral blood mononuclear cells.

A224E refers to the art-recognized CsA-resistant HIV-1 CA mutant strain.

**Overview**

The present invention is based on applicant's surprising discovery of a ligand/receptor relationship between cyclophilin ("CyP") and extracellular matrix metalloproteinase inducer ("EMMPRIN"). Moreover, anti-CD147 (*i.e.*, anti- EMMPRIN) monoclonal antibodies, which bind to the extracellular domain of the EMMPRIN), blocked CyP binding to EMMPRIN (Example 1), CyP/EMMPRIN-mediated signal transduction (Example 2) and CyPA-related HIV-1 infection/replication. Therefore, agents that modify the CyP/EMMPRIN interaction have both CyP- and EMMPRIN-related therapeutic utilities.

*Treatment and screening methods.* The present invention provides screening methods (*e.g.*, assays) to identify test compounds that modify the CyP/EMMPRIN interaction, and that thereby have therapeutic utility in methods of treatment for indications such as HIV-1 infection, acquired immune deficiency syndrome ("AIDS"), inflammatory and autoimmune disease (*e.g.*, rheumatoid arthritis ("RA"), EAE, ARDS, peridontitis), tumor growth or metastasis or any condition characterized by local or systemic CyPA release, synthesis or binding in a patient needing treatment. Compounds encompassed by the methods of present invention include, small molecules, large molecules (*e.g.*, EMMPRIN or CyP proteins, and peptides thereof) and antibodies.

Cellular and non-cellular assays can be used to identify compounds that interact with EMMPRIN, and/or CyP, to mimic, modulate or antagonize association between these molecules, and/or to modulate the activity of EMMPRIN, CyP, or the EMMPRIN:CyP complex. The cell-based assays can be used to identify compounds or compositions that affect, *e.g.*, the activity, translocation, or cellular compartmentalization of EMMPRIN, CyP, or the EMMPRIN:CyP complex, or the EMMPRIN-mediated cellular compartmentalization of other proteins (*e.g.*, HIV-1 MA and CA proteins). The assays can measure, among other parameters, direct binding to EMMPRIN, CyP, or the EMMPRIN:CyP complex , or can measure indirect effects on or by intracellular factors involved in the downstream signal transduction pathway. Such cell-based assays utilize cells, cell lines, or engineered cells or cell lines that express EMMPRIN and/or CyP proteins or peptides, or fusions thereof with unrelated proteins or peptides. The cells can be further engineered to incorporate a reporter molecule linked to the signal transduced by

EMMPRIN, CyP, or the EMMPRIN:CyP complex to aid in the identification of compounds that modulate EMMPRIN-mediated signaling activity.

EMMPRIN and CyP proteins, polypeptides and fusion proteins are used in cell, and non-cell based assays for screening compounds that interact with, and/or modulate the activity of

5    EMMPRIN, CyP, or the EMMPRIN:CyP complex, for generating antibodies, and for diagnostics and therapeutics.

EMMPRIN or CyP protein products are used therapeutically as EMMPRIN antagonists or agonists. Such EMMPRIN or CyP protein products include, but are not limited to peptides or polypeptides corresponding to one or more EMMPRIN or CyP domains (e.g., the EMMPRIN-

10   interaction domain of CyP), truncated EMMPRIN or CyP polypeptides lacking one or more EMMPRIN or CyP domains (e.g., in the case of EMMPRIN, the extracellular and/or the transmembrane domains, etc.), EMMPRIN or CyP fusion protein products (e.g., GST fusions, or epitope tagged fusions, including EGFP and FLAG fusions), and EMMPRIN or CyP protein or peptide derivatives or complexes.

15   For example, an effective amount of an appropriate soluble EMMPRIN protein or peptide, or a EMMPRIN fusion protein (e.g., EMMPRIN $^{HA}$) is administered to a patient, whereby it interacts with and alternatively "activates," "neutralizes," or "mops up" endogenous CyP, to modulate the activity of EMMPRIN, CyP, or the EMMPRIN:CyP complex. In yet another embodiment, nucleotide constructs encoding such EMMPRIN products are used to

20   genetically engineer host cells to express such EMMPRIN products in vivo. These genetically engineered cells function as "bioreactors" in the body, delivering a continuous supply of the appropriate soluble EMMPRIN product that will alternatively "activate," "neutralize," or "mop up" endogenous CyP.

Alternatively, antibodies to EMMPRIN or other agents that are EMMPRIN agonists or

25   antagonists, and/or that modulate EMMPRIN-mediated signal transduction, and that act on downstream targets in the EMMPRIN-mediated signal transduction pathway are used as therapeutic agents.


**The CyP/EMMPRIN Binding Interaction is a Novel Therapeutic Intervention Target for**

30   **Conditions Characterized by Local or Systemic CyP Release, Synthesis or Binding such as Rheumatoid Arthritis (RA)**

The CyP/EMMPRIN binding interaction is a novel intervention target for CyPA-related therapeutic indications such as RA because: **(1)** EMMPRIN is a CyP binding protein; **(2)** EMMPRIN is a signaling receptor for CyP, and mediates tyrosine kinase-dependant signal

35   transduction; and **(3)** CyP treatment activates and enhances expression and release of proMMP-1 and proMMP-3 in WI-38 primary human fibroblasts.

**(1)** *EMMPRIN was identified as a CyPA binding-protein.* Specifically, an EMMPRIN cDNA clone [SEQ ID NO:1] was isolated and initially identified as a CyPA interacting protein

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by a standard Yeast Two-Hybrid protocol (Example 1 and Figure 1). The CyPA/EMMPRIN binding interaction was then confirmed by demonstrating specific binding of [$^{35}$S]-labeled EMMPRIN to CyPA sepharose affinity resin (Example 1, Figure 1, right-hand panel).

5      (2) *EMMPRIN was identified as a signaling receptor for CyP, and mediated tyrosine kinase-dependant signal transduction*. Specifically, CyPA or CyPB induced Ca$^{2+}$ mobilization in CHO-EMM cells (that are stably transfected with an EMMPRIN-expression vector, and that constitutively express EMMPRIN), but not in control CHO cells (no EMMPRIN expression) (Example 2, and Figure 3, left-hand panel). Moreover, anti-CD147 mAb (M6FT) (*i.e.*, anti-EMMPRIN mAb), but not isotype control antibody blocked CyPA-mediated Ca$^{2+}$ mobilization

10     in CHO-EMM cells (Figure 3, right-hand panel). Furthermore, the tyrosine kinase inhibitor genistein was effective in blocking CyPA-induced signaling in CHO-EMM cells (Figure 4). Thus, EMMPRIN was identified as a cellular receptor for CyPA and CyPB, and mediated intracellular signal transduction through a tyrosine kinase-dependant pathway.

       (3) *CyPA treatment activated expression of proMMP-1, and enhanced release of*

15     *proMMP-3 in WI-38 primary human fibroblasts*. Treatment of WI-38 primary human fibroblasts with CyPA activated expression and release of the metalloproteinase zymogen proMMP-1, and enhanced the release of proMMP-3 (Example 4 and Figure 5). Metalloproteinases MMP-1 and MMP-3 are known to be elevated in the synovial fluid of RA patients, and are believed to contribute to RA disease-related pathology. Active MMP-1 and

20     MMP-3 species are processed from the corresponding secreted inactive zymogens, proMMP-1 and proMMP-3 by proteolytic enzymes normally released by surrounding tissue (*e.g.*, the proteolytic enzymes in synovial joint fluid of RA patients).

       Therefore, the mechanism by which EMMPRIN stimulates MMP production is more complex than that presented by earlier references that narrowly characterize EMMPRIN as an

25     adhesion-type molecule involved in cell-cell interactions. EMMPRIN was also identified (as disclosed herein) as a signal transducing receptor that stimulated MMP production by receiving signals via its soluble ligand, CyPA.

       Accordingly, the present invention supports a model whereby CyP released into inflammed joints during active RA disease attracts neutrophils to the joints via CyP binding to

30     EMMPRIN on neutrophil surfaces. These neutrophils are then activated in the joints via CyP-dependent, and CyP-independent mechanisms to release enzymes (*e.g.*, neutrophil elastase) that exacerbate RA disease. Therefore, anti-EMMPRIN antibodies or other agents that act as EMMPRIN agonists or antagonists have therapeutic utility for the treatment or prevention of RA or other conditions characterized by local or systemic CyP release, synthesis or binding (*i.e.*,

35     CyP-related therapeutic indications).

**The CyP/EMMPRIN Binding Interaction is a Novel Therapeutic Intervention Target for Conditions Characterized by Local or Systemic CyP Release, Synthesis or Binding such·as**

**Treatment or Prevention of HIV-1 Infection and/or AIDS or AIDS-related Disorders**

The CyPA/EMMPRIN binding interaction is a novel intervention target for HIV infection and/or AIDS or AIDS-related disorders because: **(1)** cell-surface EMMPRIN has a substantial enhancing effect on a pre-integration step of cellular infection of CHO cells by

5    MuLV-, and HIV-1-pseudotyped retroviruses carrying the HIV-1 core; **(2)** cell-surface EMMPRIN has a substantial enhancing effect on a pre-integration step of HIV infection of primary human cells (peripheral blood mononuclear cells; PBMC); and **(3)** viral resistance to the inhibitory effect of anti-CD147 mAb (UM-8D6) correlates with resistance to CsA.

**(1)** *Cell-surface EMMPRIN had a substantial enhancing effect on a pre-integration step*

10   *of cellular infection of CHO cells by MuLV-, and HIV-1-pseudotyped retroviruses carrying the HIV-1 core*. The presence of EMMPRIN protein on the cell surface of CHO cells enhanced infection by MuLV-pseudotyped HIV (Example 5 and Figure 6, left-hand panel). Moreover, this enhancement was greatly reduced by anti-CD147 mAb (UM-8D6) (Example 5 and Figure 6, left-hand panel), and such infection was blocked by EMMPRIN transmembrane domain peptide

15   1 [SEQ ID NO:3] (Example 8 and Figure 9).

Additionally, EMMPRIN enhanced CHO/CD4/CXCR4 cell (CHO cells transfected with CD4- and CXCR4-expressing vectors) infection by HIV-1 pseudotyped HIV (Example 5 and Figure 6, middle panel). Moreover, infection of PHA-activated human PBMC by Env$^{ADA}$-pseudotyped luc-HIV-1 was blocked by peptide 1 [SEQ ID NO:3] (Example 8).

20   Finally, EMMPRIN enhanced HIV infection at a stage preceding viral integration. Specifically, HIV-specific reverse transcription increased by 3- to 4-fold in the presence of EMMPRIN (*i.e.*, in CHO-EMM/CD4/CXCR4, compared to CHO/CD4/CXCR4 cells). Significantly, anti-CD147 mAb diminished this enhancing effect of EMMPRIN (Figure 6, right-hand panel), indicating that EMMPRIN acts a stage preceding viral integration.

25   **(2)** *Cell-surface EMMPRIN had a substantial enhancing effect on a post-fusion, pre-integration step of HIV infection of PBMC*. Anti-EMMPRIN mAb (UM-8D6) inhibited replication of HIV-1 strains in human primary PBMC (Example 6 and Figure 7A). Specifically, anti-CD147 mAb (UM-8D6), but not isotype-matched control mAb, inhibited replication of macrophage-tropic (ADA) and T cell line-adapted (LAV) HIV-1 strains in primary PBMC

30   (Example 6 and Figure 7A, left-hand and right-hand panels).

Significantly, anti-CD147 mAb (UM-8D6), in contrast to anti-CD4 mAb, did *not* block HIV *attachment* to human PBMC, as determined by measuring the amount of cell-bound viral p24 after incubation of the PBMC with HIV-1 at 4°C (Example 6 and Figure 7B). Therefore, anti-CD147 mAb (UM-8D6) blocked HIV-1 infection at a step between viral attachment and

35   integration.

Moreover, anti-CD147 mAb (UM-8D6), in contrast to anti-CD4 mAb, neither blocked HIV Env-mediated fusion between PBMC and HeLa cells expressing HIV-1 ADA and LAV envelopes (Example 6 and Figure 7C, left-hand panel), nor reduced cellular internalization of

20

viral p24 (Example 6 and Figure 7C, left panel). Therefore, according to the present invention, EMMPRIN acts at a post-fusion step in HIV-1 infection.

Additionally, anti CD147 mAb (UM-8D6) reduced the amount of both early and late HIV-1 reverse transcription during HIV-1 infection (Example 6 and Figure 7D). HIV-1 reverse

5    transcription is a post-fusion process that is representative of the progressive maturation of the pre-integration complex ("PIC"). Therefore, anti-CD147 mAb (UM-8D6) interfered with a step in HIV-1 infection between virus-cell fusion and initiation of reverse transcription.

Furthermore, anti-CD147 mAb (UM-8D6) blocked normal translocation of HIV-1 matrix (MA) and capsid (CA) proteins early after *de novo* infection. Specifically, in the presence of

10   anti-CD147 mAb (UM-8D6), virus was retained at the cell membrane, indicating that EMMPRIN plays a role in HIV-1 uncoating (Example 6 and Figure 7E).

(3) *Viral resistance to the inhibitory effect of anti-CD147 mAb correlated with resistance to CsA*. Replication of A224E (a CsA-resistant, CA mutant HIV strain) in human PBMC was resistant to treatment with anti-CD147 mAb (UM-8D6) (Example 7 and Figure 8).

15   Therefore, anti-CD147 mAb interfered with a CyPA-dependent step in HIV-1 infection between virus-cell fusion and initiation of reverse transcription (*i.e.*, a post-fusion, pre-integration step). Therefore EMMPRIN mediated a post-fusion, pre-integration step in HIV-1 infection (*e.g.*, viral uncoating).

Therefore, without being bound by theory, the mechanism by which CyPA mediates

20   HIV-1 infection is more complex than that presented by the prior-art, which narrowly characterizes CyPA as a protein that interacts with HIV-gag protein in virions, and with cell-surface heparans (asserted, prior to the present invention, to be the "exclusive CyPA receptor"; Saphire et al., *EMBO J.* 18:6771-6785, 1999). Surprisingly, as disclsoed herein, EMMPRIN mediated a CyPA-dependent step in HIV-1 infection. CyPA was thus involved in multiple

25   interactions with HIV-gag, heparans and EMMPRIN during HIV-1 infection. Moreover, EMMPRIN mediated the normal translocation of HIV-1 MA and CA proteins early after *de novo* infection. Demonstration that anti-CD147 mAb (UM-8D6) antagonized the novel CyPA/EMMPRIN binding interaction (and the signal transduction mediated thereby) further enables the use of other agents that modify this interaction for use in methods for the treatment

30   or prevention of HIV-1 infection and/or AIDS, and/or other conditions characterized by local or systemic CyPA release or synthesis (*i.e.*, CyPA-related therapeutic indications).

## The CyP/EMMPRIN Binding Interaction is a Novel Therapeutic Intervention Target for Conditions Characterized by Local or Systemic CyP Release, Synthesis or Binding such as
35   **Cancer**

The CyPA/EMMPRIN binding interaction is a novel intervention target for cancer because: (1) EMMPRIN-expressing tumor cells have been shown to up-regulate the expression of MMPs in fibroblasts co-cultured therewith (Biswas et al., *Cancer Res.* 55:434-439, 1995); (2)

CyP activated and/or enhances MMP production in human fibroblasts, as disclosed herein; and (3) the deregulated action of MMPs contributes to the pathological destruction of the extracellular matrix in many connective tissue diseases including, e.g., arthritis, periodontitis, tissue ulceration, and in cancer cell invasion and metastasis (Kahariet al., *Exp. Dermatol.* 6:199-
5     213, 1997; Keyszer et al., *J. Rheumatol.* 26:251-258, 1999; Benbow et al., *J. Biol. Chem.* 274:25371-25378, 1999; Keyszer et al., *Z. Rheumatology* 57:392-398, 1998).

**EMMPRIN Proteins, Polypeptides, and Antibodies**

        EMMPRIN or CyP protein and polypeptides, and mutated, truncated or deleted forms of
10    EMMPRIN or CyP and/or EMMPRIN or CyP fusion proteins can be prepared for a variety of uses, including but not limited to the generation of antibodies, as reagents in diagnostic assays, as reagents in assays for screening for therapeutic compounds that can be used in the treatment or prevention of, e.g., HIV-1 infection, AIDS, RA and cancer, and as pharmaceutical reagents useful in the treatment of, e.g., HIV-1 infection, AIDS and AIDS-related disorders, RA and
15    cancer.

        *Production of EMMPRIN and CyP Proteins and Polypeptides.* The cDNA sequence encoding EMMPRIN is shown in [SEQ ID NO:1], along with the corresponding deduced amino acid sequence [SEQ ID NO:2]. Figure 1 shows schematically the signal peptide, extracellular (ecd), transmembrane (tm) and intracellular (icd) domains of EMMPRIN. Peptides
20    corresponding to one or more domains of EMMPRIN (e.g., the EMMPRIN ecd or tm domains) or CyP, truncated or deleted EMMPRIN or CyP (e.g., EMMPRIN or CyP in which one or more regions or domains have been deleted) as well as fusion proteins in which the full-length EMMPRIN or CyP, a EMMPRIN or CyP peptide or truncated EMMPRIN or CyP is fused to an unrelated protein are also within the scope of the invention (e.g., GST, FLAG and EGFP
25    fusions). For example, such soluble EMMPRIN proteins, peptides or fusion proteins, or antibodies (including anti-idiotypic antibodies) that bind to and "activate," "neutralize" or "mop-up" circulating CyP, can be used as described herein to treat or prevent, e.g., HIV-1 infection, AIDS, RA and cancer. To this end, peptides corresponding to individual domains of EMMPRIN, soluble deletion mutants of EMMPRIN, or the entire EMMPRIN protein can be
30    fused to another polypeptide (e.g., an IgFc polypeptide, or epitope tag). Alternatively, CyP proteins, peptides or fusion proteins that act as agonists or antagonists of the EMMPRIN receptor, can be used as described herein to treat or prevent, e.g., HIV-1 infection, AIDS and AIDS-related disorders, RA and cancer.

        Such peptides, polypeptides, and fusion proteins can be prepared by recombinant DNA
35    techniques. For example, nucleotide sequences encoding one or more EMMPRIN regions or domains can be synthesized or cloned and ligated together to encode a soluble EMMPRIN protein, such as a soluble receptor or soluble binding protein or peptide (e.g., [SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5]). The DNA sequence encoding one or more EMMPRIN regions

or domains can be ligated together directly or via a linker oligonucleotide that encodes a peptide spacer. Such linkers may encode flexible, glycine-rich amino acid sequences thereby allowing the domains that are strung together to assume a conformation that can bind EMMPRIN ligands (e.g., CyPA or CyPB). Alternatively, nucleotide sequences encoding individual regions or

5 domains can be used to express EMMPRIN peptides. Similar constructions can be prepared using nucleotide sequences encoding one or more CyP regions or domains.

A variety of host-expression vector systems may be utilized to express nucleotide sequences encoding the appropriate regions of EMMPRIN or CyP to produce such polypeptides. Where the resulting peptide or polypeptide is a soluble derivative the peptide, the polypeptide

10 can be recovered from the culture media. Where the polypeptide or protein is not secreted, the EMMPRIN or CyP product can be recovered from the host cell itself.

The host-expression vector systems also encompass engineered host cells that express EMMPRIN or CyP or functional equivalents. Purification or enrichment of EMMPRIN or CyP proteins, peptides and fusion proteins from such expression systems can be accomplished using

15 appropriate methods well known to those skilled in the art. However, such engineered host cells themselves may be used in situations where it is important not only to retain the structural and functional characteristics of the EMMPRIN or CyP proteins, peptides and fusion proteins, but also to assess biological activity, e.g., in drug screening assays.

The host-expression vector systems that may be used for purposes of the invention

20 include but are not limited to microorganisms such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing EMMPRIN or CyP nucleotide sequences; yeast (e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing EMMPRIN or CyP nucleotide sequences; insect cell systems infected with recombinant virus expression vectors

25 (e.g., baculovirus) containing EMMPRIN or CyP sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing EMMPRIN or CyP nucleotide sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3, MCF-7, Hs578T) harboring recombinant expression constructs

30 containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter, or the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the EMMPRIN or CyP gene product being expressed. For

35 example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of EMMPRIN or CyP proteins, peptides and fusion proteins, or for raising antibodies to the EMMPRIN or CyP protein, vectors that direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors

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include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791, 1983), in which the EMMPRIN or CyPA coding sequence may be ligated individually into the vector in-frame with the *lacZ* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.* 13:3101-3109, 1985; Van Heeke & Schuster, *J.*

5   *Biol. Chem.* 264: 5503-5509, 1989); and the like. PGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The PGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned

10  target gene product can be released from the GST moiety.

Alternatively, utilizing an antibody or other affinity interaction specific for the fusion protein being expressed may readily allow for purification any fusion protein. For example, a vaccinia expression/affinity purification system allows for the facile expression and purification of non-denatured fusion proteins expressed in human cell lines (Janknecht, et al., *Proc. Natl.*

15  *Acad. Sci. USA* 88:8972-8976, 1991). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto $Ni^{2+}$:nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

20       *Insect systems. Autographa californica* nuclear polyhedrosis virus (AcNPV) can be used as a vector to express foreign genes in an insect system. The virus grows in *Spodoptera frugiperda* cells. The EMMPRIN- or CyP-coding sequence may be cloned individually into non-essential regions (*e.g.*, the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (*e.g.*, the polyhedrin promoter). Successful insertion of EMMPRIN- or CyP-coding

25  sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (*i.e.*, virus lacking the proteinaceous coat coded for by the polyhedrin gene). The recombinant viruses are then used to infect cells in which the inserted gene is expressed (Smith et al., *J. Virol.* 46:584, 1983; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems may be utilized. In

30  cases where an adenovirus is used as an expression vector, the EMMPRIN or CyP nucleotide sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and

35  capable of expressing the EMMPRIN or CyP gene product in infected hosts (Logan & Shenk, *Proc. Natl. Acad. Sci. USA* 81:3655-3659, 1984). Specific initiation signals may also be required for efficient translation of inserted EMMPRIN or CyP nucleotide sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire

EMMPRIN or CyP gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the EMMPRIN- or CyP-coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon,

5      must be provided. Furthermore, the initiation codon must be "in frame" with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (Bittner et al., *Methods Enzymol.*

10     153:516-544, 1987).

Additionally, a host cell strain may be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific

15     mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. Accordingly, eukaryotic host cells that possess cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include, but are

20     not limited to, CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, MCF-7, Hs578T and WI38 cell lines.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express EMMPRIN or CyP sequences may be engineered. Rather than using expression vectors that contain viral origins of replication, host

25     cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows

30     cells to stably integrate the plasmid into their chromosomes and grow to form foci that in turn . can be cloned and expanded into cell lines. This method may advantageously be used to engineer stable cell lines that express the EMMPRIN or CyP gene product (*e.g.*, the CHO-EMM cell line discussed in Example 2 below). Such engineered cell lines may be particularly useful in screening and evaluating compounds that affect the endogenous activity of the EMMPRIN gene

35     product.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., *Cell* 11:223, 1977), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA* 48:2026, 1962),

and adenine phosphoribosyltransferase (Low, et al., *Cell* 22:817, 1980) genes can be employed in tk, hgprt or aprt cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection for the following genes: *dhfr*, that confers resistance to methotrexate (Wigler et al., *Proc. Natl. Acad. Sci. USA* 77:3567, 1980; O'Hare et al., *Proc. Natl. Acad. Sci. USA* 78:1527,

5    1981); *gpt*, that confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA* 78:2072, 1981); *neo*, that confers resistance to the aminoglycoside G-418 (Colberre-Garapin et al., *J. Mol. Biol.* 150:1, 1981); and *hygro*, that confers resistance to hygromycin (Santerre et al., *Gene* 30:147, 1984).

*Antibodies to EMMPRIN or CyPA Polypeptides*. Antibodies that specifically recognize

10   one or more epitopes of EMMPRIN or CyP, or epitopes of conserved variants of EMMPRIN or CyP, or peptide fragments of EMMPRIN or CyP are also encompassed by the invention. Such antibodies include but are not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single-chain antibodies, Fab fragments, F(ab')₂ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-

15   binding fragments of any of the above.

Such antibodies are used as a method for the modulation of normal or abnormal EMMPRIN activity (*see* Examples 2, 5, 6 and 7), and are therefore useful as part of methods for the treatment and/or prevention of, *e.g.*, HIV-1 infection, AIDS, RA and cancer.

Additionally, the antibodies of the invention are used, for example, in the detection of

20   EMMPRIN in a biological sample and are, therefore, useful as part of a diagnostic or prognostic technique whereby patients or tissue samples may be tested for abnormal amounts of EMMPRIN. Such antibodies are also utilized in conjunction with, for example, compound screening schemes for the evaluation of the effect of test compounds on expression and/or activity of the EMMPRIN gene product. Additionally, such antibodies can be used in

25   conjunction with the gene therapy techniques described, below, *e.g.*, to evaluate the normal and/or engineered EMMPRIN- or CyP-expressing cells prior to their introduction into the patient.

*Antibody production*. For the production of antibodies, various host animals are immunized by injection with EMMPRIN or CyP, an EMMPRIN or CyP peptide (*e.g.*, one

30   corresponding to a functional domain of EMMPRIN or CyP, such as the transmembrane, extracellular or CyP-interaction domain), truncated EMMPRIN or CyP polypeptides (EMMPRIN or CyP in which one or more domains, *e.g.*, the transmembrane or CyP-interaction domain has been deleted), functional equivalents of EMMPRIN or CyP or mutants of EMMPRIN or CyP. Such host animals include but are not limited to rabbits, mice, hamsters and

35   rats. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially

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useful human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of the immunized animals. Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, are obtained by any technique that provides for the

5       production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique of Kohler and Milstein, (*Nature* 256:495-497, 1975; and U.S. Pat. 4,376,110), the human B-cell hybridoma technique (Kosbor et al., *Immunology Today* 4:72, 1983; Cole et al., *Proc. Natl. Acad. Sci. USA* 80:2026-2030, 1983), and the EBV-hybridoma technique (Cole et al., *Monoclonal Antibodies And Cancer Therapy*, Alan R. Liss,

10      Inc., pp. 77-96, 1985). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated *in vitro* or *in vivo*. Production of high titers of mAbs *in vivo* makes this the presently preferred method of production.

Additionally, techniques developed for the production of "chimeric antibodies"

15      (Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855, 1984; Neuberger et al., *Nature*, 312:604-608, 1984; Takeda et al., *Nature*, 314: 452-454, 1985) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those

20      having a variable region derived from a murine mAb and a human immunoglobulin constant region (humanized).

Alternatively, techniques described for the production of single-chain antibodies (U.S. Pat. No. 4,946,778; Bird, *Science* 242:423-426, 1988; Huston et al., *Proc. Natl. Acad. Sci. USA* 85:5879-5883, 1988; and Ward et al., *Nature* 334:544-546, 1989) are adapted to produce single-

25      chain antibodies against EMMPRIN or CyP gene products. Single-chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Antibody fragments that recognize specific epitopes are generated by known techniques. For example, such fragments include but are not limited to: the F(ab')$_2$ fragments, that are

30      produced by pepsin digestion of the antibody molecule; and the Fab fragments, that are generated by reducing the disulfide bridges of the F(ab')$_2$ fragments. Alternatively, Fab expression libraries are constructed (Huse et al., *Science*, 246:1275-1281, 1989) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Antibodies to EMMPRIN or CyP are, in turn, utilized to generate anti-idiotype

35      antibodies that "mimic" the EMMPRIN or CyP, using techniques well known to those skilled in the art. (Greenspan & Bona, *FASEB J.* 7:437-444, 1993; and Nissinoff, *J. Immunol.* 147:2429-2438, 1991). For example, antibodies that bind to the CyP and competitively inhibit the binding of EMMPRIN to the EMMPRIN or CyP are used to generate anti-idiotypes that "mimic" the

EMMPRIN or CyP and, therefore, bind and neutralize EMMPRIN. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes are used in therapeutic regimens to neutralize EMMPRIN.

Alternatively, antibodies are generated against EMMPRIN that act as antagonists of
5 EMMPRIN activity, *i.e.*, inhibit the activation or signaling by EMMPRIN (as shown in Example 2, *infra*). Such antibodies are used therapeutically to treat, *e.g.*, HIV-1 infection, AIDS and AIDS-related disorders, RA or cancer. Likewise, antibodies that act as agonists of EMMPRIN are generated. Such antibodies bind to EMMPRIN and activate its signal transducing activity.

10 **Screening Assays for Drugs Useful in Treating, *e.g.*, HIV-1 Infection, AIDS and AIDS-related Disorders, RA, Cancer and Conditions Characterized by Local or Systemic CyP Release, Synthesis or Binding**

Many different assay systems (*e.g.*, homogeneous, heterogeneous, cell-based and non-cell-based) can be designed and used to identify compounds or compositions that modulate
15 EMMPRIN activity or EMMPRIN gene expression, and therefore, modulate, *e.g.*, HIV-1 infection, AIDS, RA or cancer. The systems described below may be formulated into kits. To this end, the reagents (*e.g.*, EMMPRIN or CyP protein or cells expressing the EMMPRIN or CyP proteins) are packaged in a variety of containers, *e.g.*, vials, tubes, microtitre well plates, bottles, and the like. Other reagents can be included in separate containers and provided with the
20 kit; *e.g.*, positive controls samples, negative control samples, EMMPRIN or CyP proteins, peptides, buffers, cell culture media, antibodies, etc.

***Basic design and principle of screening assays.*** Without being limited to the specific embodiments discussed herein, the following assays are designed to identify compounds or compositions that, among other art-recognized possibilities: bind to EMMPRIN, CyP or the
25 CyP:EMMPRIN complex; that interfere with the interaction between CyP and EMMPRIN; or that effect EMMPRIN-mediated signal transduction or EMMPRIN-mediated cellular comparmentalization of proteins (*e.g.*, HIV-1 MA and CA proteins). Those compounds identified as antagonist or agonists of the CyP/EMMPRIN interaction, or as modifiers of EMMPRIN-mediated signal transduction or other EMMPRIN-mediated activities have
30 therapeutic utility for the treatment and prevention of, *e.g.*, HIV-1 infection, AIDS and AIDS-related disorders, RA or cancer.

The principle of the assays to identify compounds which inhibit the CyP/EMMPRIN interaction involves preparing a reaction mixture containing the test compound, functional CyP and EMMPRIN protein (or a cellular preparation comprising, in part, EMMPRIN protein), and
35 incubating the reaction mixture for a time sufficient to allow the components to interact and bind (*i.e.*, to form a complex which can be removed and/or detected). For example, to test a compound for antagonist activity, the reaction mixture is prepared in the presence and absence of the test compound. The test compound may be initially included in the reaction mixture, or

may be added at a time subsequent to the addition of CyP and EMMPRIN proteins. Control reaction mixtures are incubated without the test compound or with a control agent. The formation of any CyP:EMMPRIN complexes is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that

5    the compound antagonizes the CyP/ EMMPRIN interaction. Additionally, complex formation within reaction mixtures containing the test compound and normal CyP and EMMPRIN proteins may also be compared to complex formation within reaction mixtures containing the test compound and derivatizied or mutant CyP or EMMPRIN proteins.

*Homogeneous and heterogeneous assay formats.* Screening assays for antagonist and

10   agonists of the CyP/EMMPRIN interaction are conducted in either homogeneous or heterogeneous formats. *Homogeneous assays* are either *cell-based* or *non-cell-based*, wherein the entire reaction is carried out in a liquid phase. *Heterogeneous assays* are generally (but not always; *e.g.*, immobilized cells displaying cell-surface EMMPRIN) non-cell-based, and involve anchoring either the ligand or the receptor onto a solid phase and detecting complexes anchored

15   on the solid phase at the end of the reaction.

In either approach, the order of addition of reactants are varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the ligand/receptor interaction, *e.g.*, by competition, are identified by conducting the reaction in the presence of the test substance; *i.e.*, by adding the test substance to the reaction mixture prior

20   to or simultaneously with the ligand and interactive cellular or extracellular receptor. Alternatively, test compounds that disrupt preformed complexes, *e.g.*, compounds with higher binding constants that displace one of the components from the complex, are tested by adding the test compound to the reaction mixture after ligand/receptor complexes have been formed. The various formats are described briefly below.

25   *Heterogeneous cell-based and non-cell-based assays.* In a heterogeneous assay system, either the ligand, or the interactive cellular or extracellular receptor is anchored onto a solid surface, while the non-anchored species is labeled, either directly or indirectly. In practice, microtitre plates are utilized. The anchored species is immobilized by non-covalent or covalent attachments. Non-covalent attachment is accomplished by coating the solid surface with a

30   solution of the ligand, receptor proteins or cells expressing the receptor, and optionally drying. Alternatively, an immobilized antibody specific for the species to be anchored is used to anchor the species to the solid surface. The surfaces may be prepared in advance.

The assay is conducted by exposing the binding partner of the immobilized species to the coated surface with or without the test compound. Unreacted components are removed (*e.g.*, by

35   washing) after the reaction is complete, and any complexes formed will remain immobilized on the solid surface.

Detection of anchored complexes are accomplished in a number of ways not limited to the following. *First,* where the non-immobilized species is pre-labeled, the detection of

immobilized label indicates that complexes were formed. *Second*, where the non-immobilized species is not pre-labeled, an indirect label is used to detect anchored complexes; *e.g.*, using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending upon the order

5    of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed complexes are detected.

      *Alternatively*, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes then heterogeneously detected by, *e.g.*, using an immobilized antibody specific for

10   one of the binding components to anchor any complexes formed in solution, and detecting such anchored complexes using another labeled antibody specific for the other binding partner. Test compounds that inhibit complex formation or that disrupt preformed complexes are identified, depending upon the order of reactant addition in the initial homogeneous liquid phase.

      For example, in a heterogeneous assay the ligand or receptor proteins are first prepared

15   for immobilization using routine recombinant DNA techniques. For example, the ligand gene coding region is fused to a glutathione-S-transferase (GST) gene using a fusion vector, such as pGEX-5X-1, in such a manner that its binding activity is maintained in the resulting fusion protein. The interactive receptor is purified and used to raise a monoclonal antibody that can be labeled with the radioactive isotope $^{125}$I. The GST-ligand fusion protein is anchored to

20   glutathione-agarose beads. The interactive receptor is then added in the presence or absence of the test compound in a manner that allows interaction and binding to occur. Unbound material is washed away at the end of the reaction period, and the labeled monoclonal antibody is added to the system and allowed to bind to the complexed components. The ligand/receptor interaction is detected by measuring the amount of radioactivity that remains associated with the glutathione-

25   agarose beads. A successful inhibition of the interaction by the test compound results in a decrease in measured radioactivity.

      Alternatively, the GST-ligand fusion protein and the interactive receptor are mixed together in liquid in the absence of the solid glutathione-agarose beads. The test compound is added either during or after the species are allowed to interact. This mixture is then added to the

30   glutathione-agarose beads and unbound material is washed away. The extent of inhibition of the ligand/receptor interaction is detected by adding the labeled antibody and measuring the radioactivity associated with the beads.

      Accordingly, the present invention provides a cell-based and a non-cell based heterogeneous assay, wherein the recombinantly expressed EMMPRIN protein, polypeptide or

35   fusion protein, or CyP:EMMPRIN complex, or cells expressing the EMMPRIN receptor are attached to a solid substrate (*e.g.*, test tube, microtitre well or a column). The test compounds are then assayed for their ability to either bind to the immobilized EMMPRIN protein, polypeptide or fusion protein, or to the CyP:EMMPRIN complex, or for their ability to

antagonize or promote the binding of CyP protein, polypeptide or fusion protein to the immobilized EMMPRIN protein, polypeptide or fusion protein.

Thus, the screens are designed to identify compounds that mimic the interaction between EMMPRIN and EMMPRIN ligands, such as CyPA or CyPB. In such screens, the test

5    compounds are labeled, and are assayed for their ability to bind to immobilized EMMPRIN protein, polypeptide or fusion protein. In another aspect of the invention the screens are designed to identify compounds that antagonize the interaction between EMMPRIN and EMMPRIN ligands, such as CyPA or CyPB. In such screens, the EMMPRIN ligand is labeled and test compounds are assayed for their ability to antagonize the binding of labeled ligand to

10   EMMPRIN.

*Homogeneous cell-based and non-cell-based assays.* Homogeneous assays are either *cell-based* or *non-cell-based*, wherein the entire reaction is carried out in a liquid phase.

*Cell-based assays.* A homogeneous, cell-based assay system is used to screen for compounds that modulate the activity of EMMPRIN to identify compounds for the treatment of,

15   *e.g.,* HIV-1 infection, AIDS, RA or cancer. To this end, cells that endogenously express EMMPRIN or CyP are used to screen for compounds. Alternatively, cell lines, such as 293 cells, COS cells, CHO cells, MCF-7 cells, Hs578T cells, fibroblasts, and the like, are genetically engineered to express EMMPRIN or CyP are used for screening purposes. Preferably, host cells genetically engineered to express a functional EMMPRIN protein that responds to activation by

20   CyP or CyP peptides or fusion proteins, are used as an endpoint in the assay. Such end points, for example, are provided by measurement of art-recognized changes in chemical, physiological, biological, or phenotypic properties, such as induction of a host cell gene or a reporter gene (*e.g.,* MMPs), cAMP levels, adenylyl cyclase activity, calcium mobilization, host cell G-protein activity, host cell kinase activity, activation of signal transduction pathways, extracellular

25   acidification rate, proliferation, differentiation, susceptibility to viral infection, receptor alterations (*e.g.,* sensitization, desensitization, up- or down-regulation), etc.

To be useful in screening assays, the host cells expressing functional EMMPRIN or CyP proteins should provide for a significant EMMPRIN-based end-point response, preferably greater than 5-fold induction over background. Host cells preferably possess a number of

30   characteristics, depending on the readout, to maximize the EMMPRIN-based inductive response. For example, for detecting induction of a CRE reporter gene, a set of assay conditions comprising: (a) a low natural level of cAMP, (b) a high level of adenylyl cyclase, (c) a high level of protein kinase A, (d) a low level of phosphodiesterases, and (e) a high level of cAMP response element binding protein is advantageous. In addition, alternative pathways for

35   induction of the CRE reporter are eliminated to reduce basal levels. To increase EMMPRIN-based responses, host cells are engineered to express a greater amount of favorable factors (*e.g.,* enhanced or constitutive EMMPRIN and/or CyP expression when screening for compounds that act as EMMPRIN antagonists) or a lesser amount of unfavorable factors (*e.g.,* enhanced CyP

expression when screening for EMMPRIN agonists).

In utilizing such cell systems, the cells expressing the EMMPRIN or CyP proteins are exposed to a test compound or controls. After exposure, the cells are assayed to measure the expression and/or activity of components of the EMMPRIN signal transduction pathway.

5    Alternatively, the activity of the signal transduction pathway itself is assayed. For example, after exposure, cell lysates are assayed for calcium mobilization (*see* Example 3), induction of cAMP, or modulation of protein tyrosine kinase (PTK) (Example 3), serine/threonin kinases, g-proteins, Ras, PKA, RAP1, B-Raf, Mek, or MAPK activity or phosphorylation. Activation or deactivation of particular signal transduction pathways and upstream activators can lead to

10   activation or deactivation of many transcription factors, which in turn can mediate, among other things, cellular proliferation, differentiation, quiescence or death. This is exemplified in the neuronal differentiation of PC12 cells by PKA's phosphorylation of active sites on a small GTP-binding protein, Rap 1, and subsequent activation of a serine/threonin kinase, *B-Raf* and the MAPK kinase, *Mek* which leads to activation of MAPK. This same pathway has been

15   implicated in the NE differentiation of specific prostate cancer cell lines, such as LNCaP and PC-3M.

In screening for compounds that may act as *agonists* of EMMPRIN, it is advantageous to use cell lines that express little or no CyP to test for activation of signal transduction by the test compound as compared to controls. In screening for compounds that act as *antagonists* of

20   EMMPRIN, it is advantageous to over-express CyP, or provide exogenous CyP, to test for inhibition of signal transduction by the test compound as compared to controls.

EMMPRIN has been shown herein to mediate the normal intracellular translocation of HIV-1 matrix (MA) and capsid (CA) proteins early after *de novo* infection (Example 6 and Figure 7E). Thus, in another embodiment, test compounds are selected based on their ability to

25   regulate intracellular translocation of HIV-1 matrix (MA) and capsid (CA) proteins after viral infection.

*Non-cell-based assays*. In addition to homogeneous cell-based assays, homogeneous non-cell-based assay systems are used to identify compounds that interact with, *e.g.*, bind to EMMPRIN, CyP or to the CyP:EMMPRIN complex. Such compounds act as agonists or

30   antagonists of EMMPRIN activity and are used in the treatment of, *e.g.*, HIV-1 infection, AIDS, RA or cancer. Recombinant EMMPRIN proteins, polypeptides, fusion proteins or soluble EMMPRIN proteins are expressed and utilized (with or without CyP proteins, polypeptides or fusion proteins) in non-cell based assays to identify compounds that bind to EMMPRIN, CyP or the CyP:EMMPRIN complex. Alternatively, polypeptides corresponding to one or more

35   EMMPRIN or CyP domains, or fusion proteins containing one or more of the EMMPRIN or CyP domains are used in non-cell based assay systems to identify compounds that bind to EMMPRIN, CyP or to the CyP:EMMPRIN complex. Compounds identified are therapeutically useful to modulate EMMPRIN-mediated activities, such as signal transduction.

For example, assays based on fluorescent reporter moieties are examples of homogeneous non-cell-based assays. Such assay for example, comprise a fluorescent "reporter moiety" and a "quencher moiety," each covalently bound to linker moieties (*e.g.*, phosphoramidites, or other appropriate linkers available in the art) whereby each is attached to
5      one member of the interacting protein pair at issue (*e.g.*, a reporter moiety attached to the ligand, and a quencher moiety attached to the interacting receptor). Examples of suitable reporter and quencher molecules are 5' fluorescent reporter dyes 6FAM ("FAM"; 2,7 dimethoxy-4,5-dichloro-6-carboxy-fluorescein), TET (6-carboxy-4,7,2',7'-tetrachlorofluorescein), and the 3' quencher dye TAMRA (6-carboxytetramethylrhodamine) (Livak et al., *PCR Methods Appl.*
10    4:357-362, 1995; Gibson et al., *Genome Res.* 6:995-1001; and 1996; Heid et al., *Genome Res.* 6:986-994, 1996).

When the reporter and quencher moieties are in close proximity, the reporter moiety emission is transferred efficiently to the quenching moiety, and the fluorescent-emission spectrum (*e.g.*, at 518 nm) is quenched or masked. However, on separation, the reporter moiety
15    emission is no longer transferred efficiently to the quenching moiety, resulting in an increase of the reporter moiety fluorescent-emission spectrum. Typically, fluorochromes are chosen such that the fluorescent intensity of the quenching moiety (*e.g.*, TAMRA), changes very little over the course of the reaction. Several factor influence the efficiency of such fluorescent-based assays, including magnesium and salt concentrations, reaction conditions (time and
20    temperature), protein sequence, size and composition. Optimization of these factors to produce the optimum fluorescence intensity for a given genomic locus is obvious to one skilled in the relevant art.

Accordingly, in one embodiment embraced by the present invention, a preformed CyP:EMMPRIN complex is prepared in which either CyP or EMMPRIN (or both) is labeled
25    with a suitable fluorescent tag, whereby the signal generated by the label or tag is quenched due to complex formation (*see, e.g.*, U.S. Patent 4,109,496). The addition of a test substance that competes with and displaces one of the species from the preformed complex results in the generation of a signal above background. In this way, test substances that antagonize the CyP:EMMPRIN interaction are identified.
30

**Screening Compounds**

The assays described above are used to identify compounds that affect EMMPRIN or CyP: EMMPRIN complex activity. For example, compounds that affect EMMPRIN or CyP: EMMPRIN complex activity include but are not limited to compounds that bind to EMMPRIN,
35    inhibiting or not inhibiting binding of the natural ligand (CyP), and that either activate signal transduction (agonists) or block activation (antagonists), and compounds that bind to CyP (*e.g.*, soluble EMMPRIN proteins or peptides, or CsA derivatives) and thereby antagonize or enhance CyP-mediated EMMPRIN activity.

Compounds that modulate EMMPRIN signal transduction (*e.g.*, compounds that affect downstream signaling events, calcium mobilization (*see* Example 3), induction of cAMP, modulation of protein tyrosine kinase (PTK) (Example 3) or serine/threonin kinases, G proteins, Ras, PKA, RAP1, B-Raf, Mek, or MAPK activity or phosphorylation, which may participate in

5    transducing the signal activated by CyP binding to the EMMPRIN) are also identified by the inventive assay procedures. The present invention provides for the identification and use of such compounds that affect signaling events downstream of EMMPRIN and thus modulate effects of CyP and EMMPRIN on HIV infection, AIDS, RA and cancer.

However, it should be noted that compounds that affect EMMPRIN gene activity (by

10   affecting EMMPRIN gene expression, including molecules, *e.g.*, proteins or small organic molecules, that affect transcription or interfere with splicing events so that expression of the full-length or the truncated form of EMMPRIN can be modulated) are also identified by the inventive assay procedures.

Compounds which are screened in accordance with the invention include, but are not

15   limited to peptides, antibodies and fragments thereof, and other organic compounds (*e.g.*, peptidomimetics) that bind to EMMPRIN or the CyP:EMMPRIN complex and either mimic the activity triggered by CyP (*i.e.*, agonists) or inhibit the activity triggered by CyP (*i.e.*, antagonists); as well as peptides, antibodies or fragments thereof, and other organic compounds, including mutant or truncated EMMPRIN molecules (or a portion thereof) that bind to CyP and

20   thereby "activate" or "neutralize" CyP-mediated EMMPRIN activity. Compounds include, but are not limited to peptides, such as soluble peptides, including but not limited to members of random peptide libraries; (Lam et al., *Nature* 354:82-84, 1991; Houghten et al., *Nature* 354:84-86, 1991), and combinatorial chemistry-derived molecular libraries made of D- and/or L-configuration amino acids, phosphopeptides (including, but not limited to, members of random

25   or partially degenerate, directed phosphopeptide libraries; *e.g.*, Songyang et al., *Cell* 72: 67-778, 1993), antibodies (including, but not limited to, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, F(ab')₂ and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules. Other compounds which are screened in accordance with the invention include, but are not

30   limited to, small organic molecules that are able to cross the blood-brain barrier, gain entry into an appropriate cell and affect the expression of the EMMPRIN gene or some other gene involved in the EMMPRIN signal transduction pathway (*e.g.*, by interacting with the regulatory region or transcription factors involved in gene expression); or such compounds that affect the activity of EMMPRIN or the activity of some other intracellular factor involved in the

35   EMMPRIN signal transduction pathway, such as, *e.g.*, calcium mobilization, induction of cAMP, modulation of protein tyrosine kinase (PTK), serine/threonin kinases, G proteins, Ras, PKA, RAP1, B-Raf, Mek, or MAPK activity or phosphorylation.

Identification of the CyP/EMMPRIN interaction also enables the application of art-

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recognized computer modeling (e.g., active site modeling based on X-ray crystallography, NMR, etc.) and database searching technologies to permit identification of compounds, or the improvement of already identified compounds, that can modulate EMMPRIN expression or activity.

5

### Pharmaceutical Formulations and Methods of Treating Cancer

Compounds identified in the inventive assays that reduce or enhance CyP-mediated EMMPRIN activities, such as EMMPRIN-mediated signal transduction can be used to treat, e.g., HIV-1 infection, AIDS and AIDS-related disorders, RA or cancer. The particular method, formulation and mode of administration will depend upon the therapeutic indication, and the physico-chemical properties of the compound, and the target organ or tissue. Different approaches are discussed.

According to the present invention, treatment of therapeutically relevant EMMPRIN expressing cells in vitro with anti-CD147 mAb not only blocked infection by HIV-1, but also inhibited CyP-mediated EMMPRIN signal transduction (see Examples 2, 3, 5, 6 and 7). Therefore, antagonism of CyP-mediated EMMPRIN activity in vivo (e.g., by administration of anti-EMMPRIN antibodies, or otherwise targeting downstream signaling events) in appropriate patients would be useful in treating, e.g., HIV-1 infection, AIDS and AIDS-related disorders, RA or cancer. Alternatively, agonists of CyP-mediated EMMPRIN activity are useful, for example to enhance normal tissue remodeling processes.

It is not necessary that the therapeutic compound demonstrate absolute specificity for the CyP/EMMPRIN interaction. For example, compounds that agonize both EMMPRIN, and other unknown CyP interaction molecules, could be used. Such compounds could be administered so that delivery to joints, blood, breast tissue, the prostate, or elsewhere, is optimized to achieve, e.g., HIV-1 infection, AIDS, RA or cancer treatment, and so that potential side effects are minimized. Compounds that do not demonstrate a specificity for the CyP/EMMPRIN interaction can be administered in conjunction with another therapy or drug to control the side-effects that may result from modulating other molecules (e.g., other CyP interacting proteins); however, compounds which demonstrate a preference or selectivity for the CyP/EMMPRIN interaction are preferred.

*Dose Determinations.* Toxicity and therapeutic efficacy of such compounds are determined by standard pharmaceutical and toxicologic procedures in cell cultures or experimental animals, e.g., for determining the $LD_{50}$ (the dose lethal to 50% of the population) and the $ED_{50}$ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio $LD_{50}/ED_{50}$. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to

uninfected cells and, thereby, reduce side effects.

Data obtained from cell culture assays and animal studies is used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the $ED_{50}$ with little or no toxicity. The dosage may vary

5      within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose is typically estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the $IC_{50}$ (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as

10     determined in cell culture. Such information is used to more accurately determine useful doses in humans. Levels in plasma are measured, for example, by high performance liquid chromatography. A therapeutically effective dose further refers to that amount of the compound sufficient to inhibit HIV infection. Therapeutically effective doses may be administered alone or as adjunctive therapy in combination with other treatments for HIV infection or associated

15     diseases.

A therapeutically effective dose further refers to that amount of the compound sufficient to inhibit HIV infection, or to inhibit progression of AIDS-, RA- or cancer-related processes. Therapeutically effective doses are administered alone or as adjunctive therapy in combination with other treatments for HIV infection or associated diseases.

20     ***Formulations and Use.*** Techniques for the formulation and administration of the compounds of the instant application are found, for example, in "Remington's Pharmaceutical Sciences" Mack Publishing Co., Easton, PA, latest addition. Pharmaceutical compositions for use in accordance with the present invention are formulated in a conventional manner using one or more physiologically acceptable carriers or excipients.

25     Thus, the compounds and their physiologically acceptable salts and solvates are formulated for administration through suitable routes including, *e.g.*, inhalation or insufflation (either through the mouth or the nose), oral, buccal, rectal, transmucosal, or intestinal administration, parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal,

30     or intraocular injections, and optionally in a depot or sustained release formulation. Furthermore, one may administer the agent of the present invention in a targeted drug delivery system, for example in a liposome coated with an anti-CD4 antibody. Such liposomes are targeted to and taken up selectively by cells expressing CD4.

For oral administration, the pharmaceutical compositions take the form of, for example,

35     tablets pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, prepared by conventional means with pharmaceutically acceptable excipients such as: binding agents (*e.g.*, pregelatinised maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose,

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and/or polyvinylpyrrolidone (PVP)); fillers (*e.g.*, lactose, sucrose, mannitol, or sorbitol, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch, sodium starch glycolate, cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate); or wetting agents

5      (*e.g.*, sodium lauryl sulfate). The tablets are coated by methods well known in the art. Liquid preparations for oral administration are, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations are prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or

10     hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

15     For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*,

20     dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

25     For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers, such as Hank's solution, Ringer's solution, or physiological saline buffer.

The compounds may be formulated for parenteral administration by injection by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage

30     form in ampoules or in multi-dose containers, with an added preservative. Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or

35     liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation.

Additionally, the compounds may also be formulated as a depot preparation. Such long-acting formulations may be administered by implantation (for example subcutaneously or

5    intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (*e.g.*, as an emulsion in an acceptable oil) or ion-exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Many of the compounds of the invention identified as modulators of the CyP/EMMPRIN

10   interaction may be provided as salts with pharmaceutically compatible counterions. Pharmaceutically compatible salts may be formed with many acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc., or bases. Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms. Examples of pharmaceutically acceptable salts, carriers or excipients are well known to those

15   skilled in the art and can be found, for example, in Remington's Pharmaceutical Sciences, 18th Edition, A.R. Gennaro, Ed., Mack Publishing Co., Easton, PA, 1990. Such salts include, but are not limited to, sodium, potassium, lithium, calcium, magnesium, iron, zinc, hydrochloride, hydrobromide, hydroiodide, acetate, citrate, tartrate, malate sales, and the like.

The compositions may, if desired, be presented in a pack or dispenser device that may

20   contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

### Cell-based Gene Therapy Approaches to Controlling EMMPRIN Activity and Treating or
25   ### Preventing HIV-1 Infection, AIDS and AIDS-related Disorders, RA, Cancer or other Conditions Characterized by Local or Systemic CyP Release, Synthesis or Binding

Additional methods which may be utilized to modulate the overall level of EMMPRIN gene expression and/or EMMPRIN activity in a patient include the introduction of appropriate recombinant (genetically engineered) or non-recombinant cells that express soluble EMMPRIN

30   or CyP domains, fusion proteins thereof (*e.g.*, fusion Ig molecules of EMMPRIN or CyP), or cyclophilin (including CyP) mutants, molecular decoys or other mimics of EMMPRIN or CyP that inhibit HIV infection or EMMMPRIN-mediated signal transduction, for instance by interfering with CyP/EMMPRIN interactions. Such cells, preferably autologous cells, are administered *in vivo* at the appropriate anatomical site (*e.g.*, in the RA joint), or as part of a

35   tissue graft located at a different site in the body where they may function as "bioreactors" that deliver a supply of the soluble molecules. Such soluble EMMPRIN or CyP polypeptides and fusion proteins, when expressed at appropriate concentrations, have utility in antagonizing or agonizing endogenous EMMPRIN activity, or in neutralizing, "moping up," or "activating," as

the case may be, the native ligand for EMMPRIN (*i.e.*, CyP) and thus act as modulators of EMMPRIN activity for the treatment, *e.g.*, of HIV-1 infection, AIDS and AIDS-related disorders, RA and cancer. Such cell-based gene therapy techniques are well known to those skilled in the art (Anderson et al., U.S. Pat. No. 5,399,349; Mulligan & Wilson, U.S. Pat. No.

5   5,460,959). For example, these methods have recently been successfully applied to the treatment of human diabetes, whereby diabetic patients were rendered independent of daily insulin injections by the transplantation (engraftment) of insulin-secreting pancreatic islet cells (Shapiro et. al., *N. Engl. J. Med.* 343:230-238, 2000).

10

## Example 1

### EMMPRIN is a CyPA Interacting Protein

This example provides *in vitro* experiments using a Yeast Two-Hybrid protocol (Clontech Laboratories, Inc.; Palo Alto, CA) with CyPA as the "bait" (*i.e.*, as the interaction

15   target) to identify CyPA -interacting proteins by screening a cDNA "prey" library prepared from B lymphocytes. Specifically, the previously known transmembrane protein EMMPRIN was identified as a CyPA-interacting protein in this embodiment of the present invention.

*Methods. Yeast two-hybrid screening.* Briefly, a 'expressed sequence tag' identified as human CyPA was obtained from ATCC (ATCC #78809D). *In vitro* translation and sequencing

20   confirmed that this sequence represented a full-length CyPA cDNA (essentially homologous to GenBank #Y00052). The sequence was cloned into the vector pAS2-1 (ClonTech) to generate plasmid pAS2-1-CyPA encoding CyPA-GAL4 DNA-Binding Domain (BD) fusion protein. This plasmid was used as "bait" to screen a human B-cell cDNA library (*i.e.*, the "prey" library) (from Dr. S. Fields, SUNY, Stony Brook) constructed in pSE1107 encoding GAL4 Activation

25   Domain (AD) (library complexity ≈10⁸).

Screening was performed using the MATCHMAKER™ Two-Hybrid System 2 (Clontech Laboratories, Inc.; Palo Alto, CA) essentially as suggested by the manufacturer. In control experiments, pAS2-1-CypA did not activate transcription from the GAL4 promoter (measured as beta-galactosidase activity) when introduced alone into yeast reporter host strains (Y187 or

30   Y190), or when co-transformed with an 'empty' GAL4 Activation Domain (AD) vector (pACT2, Clontech) (negative controls). A strong positive signal was detected when pAS2-1-CypA was co-transformed with a pGAD424-GAG construct (expressing a fusion between the full-length HIV-1 *gag* protein and GAL4 AD; Luban et al., *Cell* 73:1067-1078, 1993) (positive control). The actual screening was performed by co-transforming pAS2-1-CypA together with

35   the pSE1107-cell library into the Y190 yeast reporter host strain. Selection for interacting co-transformants was performed by plating the cultures on triple drop-out plates (SD/-His/-Trp/-Leu), and then testing colonies for expression of the lacZ reporter gene by a standard filter assay protocol. All blue colonies were screened further by cycloheximide counter-selection according

to ClonTech protocols to identify false positives. Finally, plasmids from true positive colonies were isolated and sequenced.

    *The Isolated EMPRIN clone.* Approximately $10^6$ cDNA clones were screened. Ten (10) clones were selected as true positives after several rounds of screening (see under

5    "Methods" above) and sequenced. One of the clones, that interacted relatively strongly in our screen, carried an insert that was 97% identical in its sequenced 5' 186 nucleotides to human extracellular matrix metalloproteinase inducer (EMMPRIN) cDNA (DeCastro et al., *J. Invest. Dermatol.* 106:1260-1265, 1996). EMMPRIN was selected for further analysis because it was the only transmembrane protein among the CyPA-interacting clones, which made it a good

10    candidate for the role of a CyPA receptor (Figure 1). The DNA and protein sequences of EMMPRIN (human) are shown in [SEQ ID NO:1] and [SEQ ID NO:2], respectively.

    EMMPRIN is a 269-amino acid highly glycosylated cell-surface protein, having a molecular weight of 50-60 kDa, and characteristic signal peptide (sp), extracellular (ecd), transmembrane (tm) and intracellular (icd) domains (Figure 1, left panel). Structurally,

15    EMMPRIN belongs to the immunoglobulin (Ig) superfamily (Miyauchi et al., *J. Biochem. (Tokyo)* 107:316-323, 1990; Kasinrerk et al., *J. Immunol.* 149:847-854, 1992). EMMPRIN has been alternatively referred to as CD 147, basigin and M6-antigen in human (Miyauchi et al., *J. Biochem.(Tokyo)* 107:316-323, 1990; Kasinrerk et al., *J. Immunol.* 149:847-854, 1992), neurothelin and HT7 in chicken (Albrecht et al., *Brain Res.* 535:49-61, 1990), basigin and gp42

20    protein in mouse embryo (Altruda et al., *Gene* 85:445-451, 1989; Igakura et al., *Biochem. Biophys. Res. Commun.* 224:33-36. 1996), and OX-47 in rat (Fossum et al., *Eur. J. Immunol.* 21:671-679, 1991) cells. The amino acid sequences and predicted tertiary structures for all these molecules share high homology and conservation of the crucial amino acids.

    EMMPRIN is expressed on the cell surface of some tumor cells whereby, in culture, it

25    stimulates fibroblasts to produce very high levels of collagenase activity (Biswas et al., *Cancer Res.* 55:434-439, 1995). Secretion of collagenase and other metalloproteinases leads to degradation of extracellular matrix components of the basement membrane, a crucial step in tumor cell invasion and metastasis, and contributes to cartilage destruction in rheumatoid arthritis (McCachren, *Arthritis Rheum.* 34:1085-1093, 1991; Firestein, *Arthritis Rheum.*

30    39:1781-1790, 1996).

    EMMPRIN is also expressed on a wide range of normal cells. For example, EMMPRIN is known to be involved in neuronal-glial cell interactions in retinal development (Fadool & Linser, *Dev. Dyn.* 196:252-262, 1993), and in regulation of immune responses and cell-cell recognition (Berditchevski et al., *J. Biol. Chem.* 272:29174-29180, 1997). Mice lacking basigin

35    exhibit infertility of both sexes and show defects in short-term memory and learning (Igakura et al., *Biochem. Biophys. Res. Commun.* 224:33-3649, 1996). The expression of EMMPRIN is up-regulated upon T cell activation by PHA. This up-regulation leads to dimerization of EMMPRIN on the surface and makes it easily recognizable for antibodies (Edwards & Hallett,

*Immunol. Today* 18:320-324, 1997).

**Confirmation that EMMPRIN is a CyPA binding Protein**

     In another embodiment of the present invention, an affinity resin binding analysis was performed using immobilized CyPA and *in vitro* transcribed and translated [$^{35}$S]-labeled

5   EMMPRIN to confirm the EMMPRIN-CyPA interaction that was observed in the Yeast Two-Hybrid experiments. Figure 1 shows that [$^{35}$S]-labeled EMMPRIN was specifically retained by CyPA-derivatized sepharose beads (Figure 1, right-hand panel).

     *Methods. Preparation of [$^{35}$S]-labeled EMMPRIN.* EMM.C1 (containing ecd, tm and icd domains) and EMM.C2 (lacking the tm and icd domains) fragments were cloned into the

10  pT7 Blue vector and subjected to coupled *in vitro* transcription-translation reaction using a TnT-coupled reticulocyte lysate system (Promega), according to standard Promega protocols.

     *Preparation of CyPA affinity beads and binding reaction conditions.* CyPA affinity resin was prepared by coupling recombinant human CyPA to sepharose according to manufacturers instructions. In brief, one gram of freeze-dried CNBr-activated Sepharose 4B (Pharmacia;

15  Piscataway, NJ) was rehydrated in 1 mM HCl, and washed for 15 minutes with 1 mM HCl (200ml/g freeze-dried powder) on a sintered glass funnel. Washed resin was mixed with 6 mg of recombinant human CyPA dissolved in 3.3 ml of 0.1 M NaHCO3/0.5 M NaCl buffer (pH8). The mixture was rocked at room temperature for 2 hours at which time 0.15 ml of 1 M ethanolamine-HCl (pH 9.0) was added and mixture allowed to rock for two additional hours to

20  quench excess reactive groups, and then the resin was extensively washed with three cycles of alternating pH. Control resin was treated identically, except that CyPA was not added during initial coupling step. Binding reactions were performed for 90 min at room temperature in binding buffer (20 mM HEPES, pH 6.8; 150 mM KOAc; 2 mM Mg(OAc)$_2$; 2 mM DTT; 0.1% Tween 20; 0.1 mM PMSF, 1 μg/ml aprotinin, 1μg/ml pepstatin).

25      *EMMPRIN was specifically retained by CyPA-coated sepharose beads.* Aliquots of [$^{35}$S]-labeled EMMPRIN were incubated with CyPA affinity resin or with control resin. Bound proteins were eluted with standard Laemmli buffer and revealed by electrophoresis and autoradiography (Figure 1, right-hand panel). [$^{35}$S]-labeled EMMPRIN was specifically retained by CyPA sepharose beads, but not control beads lacking CyPA.

30      This specific *in vitro* binding interaction was observed only with the full-length EMMPRIN construct EMM.C2 (containing ecd, tm and icd domains), and not with the EMM.C1 EMMPRIN construct lacking the tm and icd domains. Moreover, CyPA binds to cell-surface EMMPRIN on *intact* cells and induces intracellular signaling (*see* below). These results most likely reflect a requirement for the EMMPRIN transmembrane and/or intracellular domains for

35  proper presentation of an ecd CyP-binding site to CyPA on the sepharose beads, or a mechanism whereby, CyPA directly interacts with the tm domain, with either ecd or tm domains or with both domains.

## Example 2

**EMMPRIN is a Signaling Receptor for CyPA**

   This example provides *in vitro* experiments and assays demonstrating that EMMPRIN-
5  transfected CHO cells expressed high levels of EMMPRIN on the cell surface, That CyPA and
CyPB induced $Ca^{2+}$ mobilization in such CHO-EMMPRIN cells, and that CyP-induced $Ca^{2+}$
mobilization in such CHO-EMMPRIN cells was blocked by the addition of anti-EMMPRIN
antibodies, indicating that EMMPRIN is a signaling receptor for CyP.

   The results shown in the embodiments of Example 1 (Figure 1) demonstrate that
10  EMMPRIN, expressed *in vitro*, binds to CyPA immobilized on sepharose affinity resin.
Experiments were performed to further assess whether CyP binds to EMMPRIN expressed on
the surface of cells, and whether this interaction triggers an intracellular signaling cascade.

   *CHO cell lines are EMMPRIN-negative, but were transfected to express high levels of*
*EMMPRIN.* All human, and most mouse cell lines tested already expressed EMMPRIN.
15  Therefore, CHO cells, that have no detectable EMMPRIN, were chosen to analyze molecular
mechanisms of CyP-EMMPRIN interactions. A heterogeneous population of CHO cells that
had already been transiently transfected with a human EMMPRIN expression vector was
obtained from Dr. Brian Toole (Tufts University; Boston, MA). Individual clones that stably
expressed high levels of EMMPRIN were isolated from this heterogeneous population by FACS
20  sorting using a commercially available anti-human EMMPRIN monoclonal antibody (anti-
CD147, Research Diagnostics, Inc.; Flanders, NJ). Figure 2 shows a FACS analysis of the CHO
clone (CHO-EMM) expressing the highest levels of EMMPRIN on its surface. The CHO-EMM
clone was expanded and used for all further experiments.

   *Exogenously-added CyPA or CyPB induced $Ca^{2+}$ mobilization in CHO-EMMPRIN*
25  *cells.* The relative mobilization of intracellular stores of calcium in EMMPRIN-transfected
(CHO-EMM) and control (vector-transfected) CHO cells was analyzed to determine whether the
CyP-EMMPRIN interaction leads to intracellular signal transduction.

   *Methods. $Ca^{2+}$ mobilization.* $Ca^{2+}$ mobilization assays were performed essentially as
described in Sherry et al., *Proc. Natl. Acad. Sci. U.S.A.* 95:1758-1763, 1998. Briefly, $3x10^6$
30  Fura-2-AM-loaded CHO or CHO-EMM cells ($5x10^6$ cells per ml) were stimulated with 10 µg of
human CyPA per sample, and fluorescence emission at 340 and 380 nm was measured on a
Perkin-Elmer Luminescence Spectrometer LS50B.

   CyPA induced a characteristic $Ca^{2+}$ mobilization in CHO-EMM, but not in control CHO
cells (Figure 3, left-hand panel). The results presented in Figure 3 were obtained using 10 µg/ml
35  CyPA, because additional experiments demonstrated saturation of the signaling response at this
concentration. These results demonstrate that EMMPRIN is a signal-transducing receptor for
CyPA. Similar results were obtained using CyPB in the CHO-EMM $Ca^{2+}$ mobilization assay.

   *CyP-induced $Ca^{2+}$ flux in CHO-EMMPRIN cells was blocked by the addition of anti-*

*EMMPRIN antibodies*. A calcium mobilization experiment in the presence of anti-EMMPRIN antibody, or isotype control antibody, was performed to obtain additional proof that the observed calcium flux was specifically EMMPRIN mediated (Figure 3, right-hand panel).

    *Methods. $Ca^{2+}$ mobilization*. $Ca^{2+}$ mobilization assays were performed as described
5   herein above, except that anti-CD147 mAb (M6FT) (Research Diagnostic, Inc) and isotype control antibodies (33814X, Pharmingen) were added at a concentration 500 ng/ml during the Fura-2-AM loading step.

    In the presence of anti-CD147 mAb (M6FT), CyPA did not induce $Ca^{2+}$ signaling in CHO-EMM cells (Figure 3, right-hand panel), establishing that anti-CD147 antibody and CyPA
10  binding to cell surface EMMPRIN are mutually exclusive. Additional control experiments showed that anti-CD147 mAb (M6FT) did not itself induce Ca $^{2+}$ signaling in CHO or in CHO-EMM cells. Considered together, and in view of the documented specificities of the antibodies used, these data prove that EMMPRIN is a signaling receptor for CyPA.


15                                          **Example 3**
**CyPA-induced $Ca^{2+}$ Mobilization in CHO-EMMPRIN Cells was Inhibited by Genistein**

    This example provides *in vitro* experiments and assays demonstrating that CyP-induced, EMMPRIN-mediated $Ca^{2+}$ mobilization occurs through known intracellular signal transduction pathways. CyPA-induced $Ca^{2+}$ mobilization was measured in cells pretreated with inhibitors of
20  various kinases known to be involved in the transduction of intracellular signals. Specifically, the tyrosine kinase inhibitor, genistein, completely abrogated CyPA-induced $Ca^{2+}$ flux in CHO-EMM cells that stably expressed EMMPRIN (Figure 4).

    *Methods. Inhibition of $Ca^{2+}$ mobilization analysis*. CHO-EMMPRIN cells were first pretreated for 45 minutes, during Fura-2 loading of the cells, with the following agents: Bis-
25  indolylmaleimide I (10 nM), a PKC inhibitor; PD98059 (100 μM), a MAPK inhibitor; and genistein (67 μM), a tyrosine kinase inhibitor. The cells were then stimulated with 10 μg/ml CyPA, and subjected to $Ca^{2+}$ mobilization analysis as described herein above. Alternatively, *Bordetella pertussis* toxin (PTX; at 100 ng/ml), which inactivates signaling pathways mediated by members of the Gi-Go and Gt-protein family, or the PTX B-oligomer (100 ng/ml), which was
30  recently shown to induce cross-desensitization of chemokine receptors (Alfano et al., *J. Exp. Med.* 190:597-606, 1999), were added 12 hr prior to stimulation with 10 μg/ml CyPA and $Ca^{2+}$ mobilization analysis.

    *Genistein completely abrogated CyPA-induced, EMMPRIN-mediated $Ca^{2+}$ mobilization in CHO-EMM cells*. Figure 4 shows that Bis-indolylmaleimide I (a PKC
35  inhibitor) and PD98059 (a MAPK inhibitor) had no effect on CyPA-induced $Ca^{2+}$ mobilization in CHO-EMM cells. Likewise, treatment of cells with pertussis toxin (PTX) or the PTX B-oligomer failed to block CyPA-induced $Ca^{2+}$ signaling in CHO-EMM cells. In contrast, genistein, an inhibitor of tyrosine kinases, completely abrogated $Ca^{2+}$ mobilization in CyPA-

stimulated CHO- EMMPRIN cells (Figure 4, lower curve). These data show that CyPA-induced, EMMPRIN-mediated signal transduction occurs through a tyrosine kinase-dependent pathway.

5                                        **Example 4**
**CyPA-treatment Up-regulated proMMP Expression and Release by Cultured Fibroblasts**

   This example provides *in vitro* experiments and assays to determine whether CyP-treatment up-regulates matrix metalloproteinase (hereinafter "MMP") expression in primary lung fibroblasts. The present embodiment demonstrates that CyPA-treatment activated
10  expression and release of MMP-1, and enhanced that of MMP-3 by WI-38 cultured human primary lung fibroblasts (Figure 5).

   *Methods. CyP-treatment of primary human lung fibroblasts; western immuno-blot analysis.* First, primary WI-38 human lung fibroblasts (which express high levels of EMMPRIN) were grown to 80% confluence, serum-starved overnight, and then either incubated
15  alone, or in the presence of 100 ng/ml CyPA, for five (5) hours. Cell supernatants and membranes were then collected, and analyzed for MMP expression by Western immuno-blot analysis using specific mouse antibodies (Cal Biochem; La Jolla, CA).

   *CyPA treatment activated expression and release of proMMP-1, and enhanced expression and release of proMMP-3 by WI-38 fibroblasts.* The question of whether CyPA
20  induces the expression of MMP-1, MMP-3, and MMP-9 was examined, because these three MMPs have been reported to be elevated in the synovial fluid of rheumatoid arthritis patients, and are believed to contribute to disease-related pathology (Sorsa et al., *Semin. Arthritis Rheum.* 22:44-53, 1992; Keyszer et al., *J. Rheumatol.* 26:251-258, 1999; So et al., *Rheumatology (Oxford).* 38:407-410, 1999; McCachren, *Arthritis Rheum.* 34:1085-1093, 1991).
25  Figure 5 shows that WI-38 fibroblasts treated with CyPA, but not untreated fibroblasts, expressed and released MMP-1 into the culture fluid. Additionally, CyPA enhanced the expression and release of MMP-3, which was otherwise constitutively secreted by these cells. MMP-9 was not detected in the supernatant fluids of untreated or CyPA-treated cells.

   Furthermore, based upon molecular weights as assessed by SDS-PAGE analysis, it was
30  determined that the pro-enzyme forms of MMP-1 and MMP-3 were released in response to CyPA. Parallel experiments analyzing active MMPs using zymography did not reveal bands at 45 kDa and 33 kDa that would correspond to mature, active MMP-1 and MMP-3, respectively. This was not surprising because MMP expression was examined according to the present embodiment in purified populations of resting fibroblasts in culture. All members of the MMP
35  family are known to be secreted as inactive zymogens (*e.g.*, proMMP-1 and proMMP-3) that are subsequently activated extracellularly by proteolytic enzymes released by surrounding cells and tissue (Woessner, *FASEB J.* 5:2145-2154, 1991). The inflamed RA joint, relative to a normal joint, contains a marked increase in the number of neutrophils thought to be the source of

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enzymes which ultimately cleave proMMP-1 and proMMP-3 to their active forms. Activated MMPs then act to degrade cartilage-leading to joint destruction (Edwards & Hallett, *Immunol. Today* 18:320-324, 1997).

These data support a dual mechanism(s) for EMMPRIN-mediated stimulation of matrix
5   metalloproteinase activity *in vivo*, whereby EMMPRIN both receives (*i.e.*, as a signal-transducing cellular receptor) a signal via an extracelluar CyP ligand, and imparts (*i.e.*, as an anchored, cell-surface "ligand") a signal via cell-cell adhesion-type interactions with a counter receptor on another cell.

10                                              **Example 5**
**Cell-surface EMMPRIN had a Substantial Enhancing Effect on a Pre-integration Step of Cellular Infection of CHO cells by MuLV-pseudotyped Retroviruses Carrying the HIV-1 Core**

This example provides *in vitro* experiments and assays showing, in CHO cells, that
15   EMMPRIN is involved in HIV infection. Specifically, according to the present embodiment, the presence of cell-surface EMMPRIN had a substantial enhancing effect on a pre-integration step of cellular infection by MuLV-pseudotyped retroviruses carrying the HIV-1 core (Figure 6).

*Methods. CHO cell lines.* CHO cells, that do not express EMMPRIN, were initially used in DNA transfection experiments to investigate the role of EMMPRIN in HIV-1 infection.
20   This was because all potentially HIV-1-susceptible human cells that were tested (primary T cells and macrophages, T lymphocytic and monocytic, HOS, 293T, and HeLa cell lines) already expressed relatively high levels of EMMPRIN. Several CHO-EMM clones were selected (*see* Example 2) from EMMPRIN-transfected CHO cells that were provided by Dr. Brian Toole, Tufts University, Boston, MA, and that expressed high levels of EMMPRIN. No EMMPRIN
25   was detected in control (pcDNA-transfected) CHO cells.

*Transfection of CHO cells.* CHO cells grown to 80% confluency in 75cc flasks were transfected with 10 µg of CD4 or CXCR4 expression vectors (pBABE-T4 and pBABE-CXCR4, respectively) using Fugene (Boehringer Mannheim) and following the manufacturer-provided protocol. The transfection efficiency was typically 15-20%, as revealed by flow cytometric
30   analysis.

*Antibodies.* Anti-CD147 mAb (UM-8D6) was obtained from Ancell Immunology Research Products (Bayport, MN), and isotype-matched (IgG1κ) control mAb was from BD PharMingen (San Diego, CA). Anti-CD4 mAb was from Beckton Dickinson (Parsippany, NJ).

*Infection with luciferase-expressing pseudotyped HIV-1 variants.* Single-cycle assays
35   with Env-pseudotyped, luciferase-expressing HIV-1 recombinants were performed essentially as previously described (Connor et al., *J. Exp. Med.* 185:621-628, 1997; Dragic et al., *Nature* 381:667-673, 1996). Briefly, pseudotyped luciferase reporter viruses were produced by co-transfection of 293T cells with a plasmid expressing an envelope-deficient NL4-3 construct,

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along with a plasmid expressing viral envelopes derived from either the X4 strain HxB2, or the amphotropic murine leukemia virus (MuLV). Cells infected with pseudotyped virus (5 ng of p24 per $10^6$ cells) were lysed using reporter lysis buffer (Promega) after 4 days in culture, and luciferase activity was measured in relative light units using a Dynex MLX microplate

5     luminometer.

*EMMPRIN enhanced CHO cell infection by MuLV pseudotyped HIV.* A luciferase activity analysis of infection of CHO-EMM or control CHO cells with recombinant luciferase-expressing HIV-1 pseudotyped with an amphotropic envelope of murine leukemia virus (MuLV) was performed to determine whether EMMPRIN enhanced HIV infection.

10     Triplicate cultures of CHO-EMM or control (pcDNA-transfected) CHO cells were infected, in the presence or absence of 50 μg/ml of anti-CD147 (*i.e.,* anti-EMMPRIN) mAb or isotype-matched control mAb, with luciferase-expressing HIV-1 (Luc-HIV-1) pseudotyped with Env derived from MuLV. Heparinase III ($3 \times 10^{-4}$ IU/ml) was added to cells prior to infection for 1 hr at 37°C. Luciferase expression was measured on day 4 post-infection and is presented as

15     percentage of expression relative to control (CHO-pcDNA cells) taken as 100%.

Cell-surface EMMPRIN increased luciferase expression 5- to 6-fold in CHO cells (Figure 6, left-hand panel). Moreover, this increase was greatly reduced when anti-CD147 mAb was added during infection (Figure 6, left-hand panel, third vertical bar).

*EMMPRIN enhanced CHO cell infection by HIV-1 pseudotyped HIV.* CHO-EMM or

20     control CHO cells were transiently transfected with CD4- and CXCR4-expressing vectors, and then infected with a luciferase-expressing HIV-1 construct pseudotyped with the LAV envelope to determine whether EMMPRIN enhanced replication of viruses pseudotyped with HIV-1 envelope.

Triplicate cultures of CHO or CHO-EMM cells were transiently transfected with CD4-

25     expressing pBABE-T4 and CXCR4-expressing pBABE-CXCR4 and infected with Luc-HIV-1 pseudotyped with Env derived from HIV-1$^{LAV}$, which depends on CD4 and CXCR4 for entry. Luciferase expression was detected as described herein above, and is presented as percentage of expression relative to control cells (CHO/CD4/CXCR4) taken as 100%.

Like the above results with the MuLV envelope, a 3- to 4-fold increase in luciferase

30     expression was observed in cells expressing EMMPRIN (Figure 6, middle panel).

*EMMPRIN enhanced HIV infection at a stage preceding viral integration.* The amount of HIV-1 reverse transcription was measured in the presence or absence of anti-CD147 mAb in infected CHO cells to determine if the enhancing effect of EMMPRIN on HIV infection was manifested at a stage preceding integration of the proviral DNA.

35     Duplicate cultures of CHO and CHO-EMM cells were transiently transfected with CD4- and CXCR4-expressing vectors, and then infected with luc-HIV-1 pseudotyped with either Env$^{MuLV}$ or Env$^{LAV}$ in the presence of anti-CD147 mAb (UM-8D6) or isotype-matched control antibody. Viral DNA was analyzed by PCR two hours after infection using LTR R/U5 primers

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[SEQ ID NO:2] [SEQ ID NO:3], and probe [SEQ ID NO:4] specific for early reverse transcription products (Schmidtmayerova et al., *J. Virol.* 72:4633-4642, 1998). Results were quantified on a Direct Imager (Packard) and are presented as radioactivity associated with LTR/*gag*-specific signal relative to control cells (isotype-treated CHO/CD4/CXCR4) taken as 100%.

The amount of LTR R/U5 reverse transcription product following infection with Env$^{MuLV}$- and Env$^{LAV}$-pseudotyped viruses was increased 3- to 4-fold in CHO-EMM/CD4/CXCR4 cells compared to CHO/CD4/CXCR4 cells (Figure 6, right-hand panel). Moreover, anti-CD147 mAb (UM-8D6) diminished this enhancing effect of CD147 (Figure 6, right-hand panel, last vertical bar in each series), consistent with the anti-CD147 mAb (UM-8D6) effect described above on luciferase expression. The reason for the incomplete suppression of CD147 activity by anti-CD147 mAb (UM-8D6) likely resulted from the high level of EMMPRIN expression in CHO-EMM cells, and/or from differences in the EMMPRIN epitopes recognized by the antibody compared to virus-associated CyPA.

Therefore, EMMPRIN had an enhancing effect on a pre-integration step of cellular infection by retroviruses carrying the HIV-1 core. Significantly, this effect did not depend on the identity of Env used for pseudotyping. Thus, the activity of EMMPRIN in enhancing HIV-1 infection does not appear to depend on the nature of receptors used for entry. Such a receptor-independent mode of action resembles the activity of CyPA, which is reported to mediate *attachment* of pseudotyped HIV-1 core-containing viruses through interaction with heparans (Saphire et al., *EMBO J.* 18:6771-6785, 1999). However, heparans are not required for the enhancing effect of EMMPRIN on replication of luciferase-expressing Env$^{MuLV}$-pseudotyped HIV-1. Treatment of CHO-EMM cells with heparinase III, which significantly reduced HIV-1 attachment (Figure 7B), did not diminish EMMPRIN-mediated luciferase activity (Figure 6, left-hand panel).

Therefore, the instant data are consistent with a mechanism whereby heparans, while potentially involved in gp120-mediated attachment (Mondor et al., *J. Virol.* 72:3623-3634, 1998), do not contribute to the EMMPRIN-mediated enhancing effect on a HIV-1 infection step preceding viral integration.

## Example 6
**Cell-surface EMMPRIN had a Substantial Enhancing Effect on a Post-fusion, Pre-integration Step of HIV-1 Infection of Primary Human PBMC**

This example provides *in vitro* experiments and assays showing that EMMPRIN is involved in HIV infection of human primary peripheral blood mononuclear cells (PBMC). Specifically, the presence of cell-surface EMMPRIN on PBMC had a substantial enhancing effect on a post-fusion, pre-integration step of cellular infection by T cell-adapted or

macrophage-tophic HIV-1 strains, or by pseudotyped retroviruses carrying the HIV-1 core
(Figure 7A-E).

   *Methods. Preparation and infection of peripheral blood mononuclear cells (PBMC).*
Human primary peripheral blood mononuclear cells (PBMC) were obtained from whole blood
5   using standard Ficoll-Hypaque gradient centrifugation methods. Cells were activated with PHA
(5 µg/ml) for 2 days and were then infected with HIV-1$^{LAV}$ or HIV-1$^{ADA}$ (5x10$^3$ cpm of RT
activity per 1x10$^6$ cells) in the presence of various concentrations of anti-CD147 mAb (UM-
8D6) or isotype-matched control mAb. Virus replication was assayed by measuring RT activity
in culture supernatants.

10   *Infection with luciferase-expressing pseudotyped HIV-1 variants.* Preparation of, and
infection with pseudotyped Luc-HIV-1 strains was performed as described above under Example
5, except that the envelopes were derived from either LAV or ADA strains.

   *Virus attachment assay.* PHA-activated PBMC were incubated for 2 hours at 37°C with
anti-CD147 mAb (UM-8D6; at 50 µg/ml), anti-CD4 mAb (5 µg/ml), or heparinase III (3x10$^{-4}$
15   IU/ml), and then washed and incubated for one hour at 4°C with HIV-1$^{LAV}$ (10 ng/ml of p24).
Antibodies, but not heparinase, were present during the one-hour incubation periods at 4°C with
HIV-1$^{LAV}$. The amount of cell-bound viral p24 was assayed by ELISA after extensive washing
of the cells.

   *Fusion assays. Cell-cell fusion.* HIV-1 Env-mediated cell fusion was assayed using a
20   colorimetric fusion technique (Alkhatib et al., *J. Virol.* 70:5487-5494, 1996). Briefly, primary
PHA-activated PBMC were infected with vaccinia virus expressing T7 RNA polymerase (vTF7-
3, Fuerst et al., *Proc. Natl. Acad. Sci. U.S.A.* 83:8122-8126, 1986), and co-incubated in the
presence of anti-CD147 mAb (UM-8D6) or isotype-matched control mAb for 2.5 hr at the ratio
of 5:1 with HeLa cells co-infected with vaccinia viruses expressing HIV-1 Env (vCB-41 for
25   expressing X4 Env$^{LAV}$, and vCB-43 for expressing R5 Env$^{Ba-L}$; Broder & Berger, *Proc. Natl.
Acad. Sci. U.S.A.* 92:9004-9008, 1995) and *E. coli lacZ* gene linked to the T7 promoter (vCB-
21R-lacZ, Alkhatib et al., *J. Virol.* 70:5487-5494, 1996) to allow cell fusion. A standard
colorimetric assay, measuring β-Gal activity, was used with detergent cell lysates for assessing
cellular fusion. The results obtained were expressed as β-Gal activity relative to a positive
30   control (no antibody treatment, taken as 100%), and presented below as the mean ± SD of three
independent measurements with cells from the same donor.

   *Virus-cell fusion.* For analysis of virus-cell fusion, PHA-activated PBMC were
incubated with HIV-1$_{LAV}$ for 2 hr at 37°C in the presence or absence (*i.e.,* "control") of the
indicated antibody. Cells were treated with trypsin (0.025% for 30 min at 37°C), lysed, and the
35   internalized viral p24 was measured in the lysates by ELISA. The background of this assay (*i.e.,*
the 4°C "control") was determined by incubating cells at 4°C with the virus but without
antibody, and then transferring them to 37°C in the presence of trypsin. This value reflects the
amount of virus that manages to enter cells in the presence of trypsin.

*Detection of HIV-1 specific DNA by PCR (Reverse Transcriptase assay).* PHA-activated human PBMC were treated with either anti-CD147 mAb (UM-8D6; at 50 µg/ml), anti-CD4 mAb (2 µg/ml), or with the corresponding isotype-matched control mAb (50 µg/ml) 2 hr before infection with HIV-1. Analysis of HIV-1 reverse transcription was then performed 2 hr after
5  infection of PBMCs with HIV-1<sup>LAV</sup> using the indicated primers, essentially as described in Schmidtmayerova et al., *J. Virol.* 72:4633-4642, 1998.

Briefly, cells were lysed in standard PCR buffer with proteinase K. The proteinase K was heat inactivated, and 25 µl aliquots were subjected to 35 cycles of PCR in a total volume of 50 µl containing 0.2 µM oligonucleotide primers, 200 µM of each deoxynucleotide, 50 mM
10  KCl, 10 mM Tris (pH 8.3), 2 mM MgCl$_2$, and 1.25 U of *Taq* DNA polymerase (Perkin-Elmer). Each cycle comprised a 30-sec. denaturation step at 94°C, a 30-sec. annealing step (Tm-5°C for each pair of primers), and a 1-min. extension at 72°C. After agarose gel electrophoresis, amplified DNA was analyzed by Southern blot hybridization with the [$^{32}$P]-labeled probe. Amplified fragments of the correct size were then quantified with an Instant Imager (Packard)
15  and expressed as a percentage of counts in antibody-treated, relative to antibody-untreated controls (taken as 100%). The following primers and probes were used: LTR R/U5, sense primer (5'-GGCTAACTAGGGAACCCACTG-3') [SEQ ID NO:6], antisense primer (5'-CTGCTAGAGATTTTCCACACTGAC-3') [SEQ ID NO:7], and probe (5'-TGTGTGCCCGTCTGTTGTGTG-3') [SEQ ID NO:8]; LTR/gag primers, sense primer (5'-
20  CAGATATCCACTGACCTTTGG-3') [SEQ ID NO:9], antisense primer (5'-GCTTAATACTGACGCTCTCGCA-3') [SEQ ID NO:10], and probe (5'-GAGGCTTAAGCAGTGGGTTC-3') [SEQ ID NO:11] (*see* Schmidtmayerova et al., *supra*).

*Subcellular fractionation and Western blot analysis.* Subcellular fractions of MT-4 cells infected with HIV-1$_{LAI}$ were prepared using a previously published protocol (Bukrinskaya et al.,
25  *J. Exp. Med.* 188:2113-2125, 1998) with some modifications. Briefly, cells were pelleted and incubated on ice in a hypotonic buffer (10 mM HEPES, pH 6.9, 10 mM KCl, 0.1 mM PMSF and 1 µg/ml aprotinin) for 15 min. Cells were then disrupted by Dounce homogenization, and nuclei were pelleted at 1,500 x g for 5 min. and discarded. The supernatant fraction was removed and centrifuged at 18,000 x g for 45 min. Supernatant from this second centrifugation was reserved,
30  and is referred to herein as the "cytosolic" fraction. The pellet from the second centrifugation, containing the cytoskeleton and membrane fractions, was resuspended in NTENT buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7.2, 1 mM EDTA, 1% Triton X-100, 0.1 mM PMSF and 1 µg/ml aprotinin) supplemented with 1% n-octyl-β-D-glycopyranoside, and was centrifuged at 18,000 x g for 30 min. The supernatant from this third centrifugation was reserved, and is
35  referred to herein as "membrane" fraction, whereas the corresponding pellet represents the "cytoskeleton" fraction. Subcellular fractions from an equivalent of 2x10$^6$ cells/lane were fractionated on a 12% SDS-PAGE and analyzed by Western blot assay using monoclonal antibodies to MA, CA (both from ABI), and actin (Sigma).

49

*Anti-EMMPRIN mAb inhibited replication of HIV-1 strains in human primary*
*PBMC.* Anti-CD147 (*i.e.*, anti-EMMPRIN) mAb (UM-8D6) was used to further investigate the
mechanism of EMMPRIN activity in primary human PBMC (Figure 7A).

Triplicate cultures of PHA-activated PBMC were infected with either the T cell line-
5   adapted X4 HIV-1$_{LAV}$ strain or the macrophage-tropic R5 HIV-1$_{ADA}$ strain (Figure 7A, left
panel), or with a luciferase-expressing recombinant HIV-1 pseudotyped with envelopes derived
from either LAV or ADA strains (Figure 7A, right panel). Anti-CD147 mAb (UM-8D6) in the
amounts indicated (50 µg/ml was used for the experiment in the right panel) or isotype-matched
control mAb (50 µg/ml in all control samples) was added 30 minutes prior to infection and was
10  present throughout the duration of the experiment. Virus replication was assessed by RT activity
in culture supernatants on day 21 (Figure 7A, left panel), or by luciferase activity on day 4 after
infection (right panel). Results are presented as percentages of RT (using LTR R/U5 primers
[SEQ ID NO:6] and [SEQ ID NO:7], and probe [SEQ ID NO:8]) or luciferase activity relative
to controls (cells treated with isotype-matched irrelevant mAb; taken as 100%).

15  Anti-CD147 mAb (UM-8D6), but not isotype-matched control mAb, inhibited
replication of macrophage-tropic (ADA) and T cell line-adapted (LAV) HIV-1 strains in primary
PBMC (Figure 7A, left panel). A similar result was observed with recombinant luciferase-
expressing viruses pseudotyped with ADA or LAV envelopes (Figure 7A, right panel).

*Anti-CD147 mAb (UM-8D6) did not block HIV attachment to human PBMC.* The
20  effect of anti-CD147 mAb (UM-8D6) on different early steps of HIV-1 replication (*e.g.*, viral
attachment, fusion, uncoating, etc.) in PBMC was analyzed to define the mechanism of its
inhibitory activity on HIV infection (Figure 7B).

The viral attachment step was analyzed by incubating virus particles with the cells at 4°C,
and then measuring the amount of cell-bound viral p24. Specifically, triplicate PBMC cultures
25  were pre-treated at 37°C with anti-CD147 mAb (UM-8D6; at 50 µg/ml), anti-CD4 (5 µg/ml)
mAb, or heparinase III (3x10$^{-4}$ IU/ml), and then washed and incubated with HIV-1$^{LAV}$ (10 ng/ml
of p24) at 4°C for 1 hr as described in this Example under "methods," above. The amount of
cell-bound p24 was assayed by ELISA, and the results are shown for one representative
experiment out of three performed with cells from different donors.

30  Significantly, anti-CD147 mAb (UM-8D6) did not block HIV-1$^{LAV}$ attachment to human
PBMC, whereas anti-CD4 mAb significantly reduced it (Figure 7B). As expected, HIV-1$^{LAV}$
attachment was also reduced by pre-treatment of the cells with heparinase III, in accordance with
the postulated role of heparans in HIV-1 attachment (Saphire et al., *EMBO J.* 18:6771-6785,
1999 (Host CyPA mediates HIV-1 attachment to target cells via heparans); Mondor et al., *J.*
35  *Virol.* 72:3623-3634, 1998 (HIV-1 attachment to HeLa CD4$^+$ cells is CD4-independent, gp120
dependent and requires cell-surface heparans). Therefore, these data show that anti-CD147 mAb
(UM-8D6) blocked HIV infection at a step somewhere between viral attachment and integration.

*Anti-CD147 mAb (UM-8D6) neither blocked HIV Env-mediated fusion between PBMC and HeLa cells expressing HIV-1 ADA and LAV envelopes, nor reduced cellular internalization of viral p24.* The effect of anti-CD147 mAb (UM-8D6) on HIV-1 Env-mediated fusion between PBMC and HeLa cells expressing HIV-1 ADA and LAV envelopes was

5      analyzed to determine if EMMPRIN is involved at the viral-cell fusion step of replication · (Figure 7C, left panel). Additionally, the effect of anti-CD147 mAb (UM-8D6) on fusion in the context of HIV-1 infection was analyzed by measuring the amount of viral p24 that remained associated with HIV-1$^{LAV}$-inoculated PBMC after trypsinization (*i.e.*, that corresponding to internalized p24) (Figure 7C, right panel).

10     *Cell-cell fusion.* Fusion between PBMC and HeLa cells expressing HIV-1 Env (X4 LAV or R5 Ba-L) was assessed in the presence of anti-CD147 (UM-8D6; at 50 μg/ml) or anti-CD4 (5 μg/ml) mAb by β-Gal expression as described herein above under "Methods" in this Example. The background activity detected in co-cultures of T7 RNA polymerase-expressing PBMC with HeLa cells expressing only the *E. coli LacZ* gene linked to the T7 promoter was subtracted from

15     all values. The anti-CD147 mAb (UM-8D6) did not inhibit HIV-1 env-mediated cell-cell fusion, whereas, as expected, the anti-CD4 mAb significantly reduced cell fusion (Figure 7C, left panel).

*Virus-cell fusion.* For analysis of virus-cell fusion, PBMC were incubated with HIV-1$^{LAV}$ as described herein under "Methods" in this Example, *supra*. The anti-CD147 mAb (UM-8D6)

20     did not inhibit viral p24 internalization, whereas, as expected, the anti-CD4 mAb reduced the amount of internalized p24 to background levels (Figure 7C, right panel). Thus, anti-CD147 antibody did not reduce the amount of viral proteins that became resistant to trypsin digestion as a result of virus-cell fusion.

Therefore, the collective results of Figure 7C (both panels) indicate that the inhibitory

25     effect of anti-CD147 mAb on HIV-1 infection is mediated by a post-fusion mechanism.

*Anti CD147 mAb (UM-8D6) reduced the amount of both early and late HIV-1 reverse transcription.* A semi-quantitative PCR method was used to analyze the amount of HIV-1-specific reverse transcription, a post-fusion process that is representative of the progressive maturation of the pre-integration complex (PIC) (Figure 7D).

30     PHA-activated human PBMC were treated with either anti-CD147 mAb (UM-8D6; at 50 μg/ml), or anti-CD4 mAb (2 μg/ml), or with the corresponding isotype-matched control mAb (50 μg/ml) 2 hr before infection with HIV-1. Analysis of HIV-1 reverse transcription was then performed 2 hr after infection of PBMCs with HIV-1$^{LAV}$ as described above under "Methods," using primers LTR R/U5 [SEQ ID NO:6] and [SEQ ID:NO:7], and probe [SEQ ID NO:8]

35     specific for the "strong-stop" early reverse transcription products (Schmidtmayerova et al., *J. Virol.* 72:4633-4642, 1998). The PCR results were quantified on a Direct Imager (Packard) and are presented as percentage of counts in treated, relative to antibody-untreated control (taken as 100%). Dilutions of 8E5/LAI cells containing one HIV-1 genome per cell (Folks et al., *J. Exp.*

*Med.* 164:280-290, 1986) were used as PCR standards (Figure 7D, lower-left panel). The data are representative of three independent experiments, performed in duplicate.

The anti-CD147 mAb (UM-8D6) reduced the amount of the "strong-stop" reverse transcription product by approximately 2-fold (Figure 7D, upper-left and right-hand panels).

5    This short DNA fragment is produced early after virus entry and its amount reflects initiation of reverse transcription (Zack et al., *Cell* 61:213-222, 1990). As expected, anti-CD4 mAb reduced the amount of LTR R/U5-amplified fragment even more dramatically (Figure 7D, upper-left and right-hand panels), consistent with the herein-described effect of this mAb on virus attachment and virus-cell fusion (Figures 7B and 7C). A similar effect of anti-CD147 mAb (UM-8D6) was

10   observed on the amount of PCR fragment detected with LTR/*gag* primers [SEQ ID NO:9] and [SEQ ID NO:10], and probe [SEQ ID NO:11], which amplify and detect, respectively, a late-stage reverse transcription product (Schmidtmayerova et al., *J. Virol.* 72:4633-4642, 1998).

Therefore, the results of the present embodiment, together with data presented herein in Figures 7A, 7B and 7C, show that anti-CD147 mAb (UM-8D6) interfered with a step between

15   virus-cell fusion and initiation of reverse transcription. One likely mechanism of such activity, without being limited to any specific theory, is inhibition of pre-integration complex (PIC) formation, whereby CD147 plays a role in HIV-1 uncoating.

*Anti CD147 mAb (UM-8D6) blocked normal translocation of HIV-1 matrix (MA) and capsid (CA) proteins early after de novo infection, indicating that EMMPRIN was involved in*

20   *HIV-1 uncoating.* Virus "uncoating" occurs soon after HIV infection, and involves dissociation of viral capsid protein (CA) from the viral matrix protein (MA) and nucleoprotein complex (Bukrinsky et al., *Proc. Natl. Acad. Sci. U.S.A* 90:6125-6129, 1993; Miller et al., *J. Virol.* 71:5382-5390, 1997). Specifically, MA relocates from membranes to the cytoskeleton, while CA is retained in the cytosol (Boukrinskaya et al., *J. Exp. Med.* 188:2113-2125, 1998). This

25   pattern of protein rearrangements correlates with association of the HIV-1 reverse transcription complex with the cytoskeleton and suggests involvement of the cytoskeleton in the early steps of PIC formation (Bukrinskaya et al., *J. Exp. Med.* 188:2113-2125, 1998).

The intracellular distribution of HIV-1 matrix (MA) and capsid (CA) proteins early after *de novo* infection was investigated to determine whether EMMPRIN was involved in the HIV-1

30   "uncoating" step during infection (Figure 7E).

MT-4 cells were inoculated at 4°C with HIV-1[LAI] in the presence of anti-CD147 mAb (UM-8D6; at 50 µg/ml) or isotype-matched control mAb. An aliquot (1x10^6 cells) was withdrawn after 30 minutes for protein analysis (*i.e.*, the 0 hr p.i. time-point sample) (Figure 7E, upper panel), while the inoculated cultures were transferred to 37°C and incubated for 1.5 hr

35   (Figure 7E, lower panels). Subcellular fractionation was performed as described herein above in this Example under "Methods," and proteins in cytosolic (C), membrane (M), and cytoskeleton (CS) fractions were analyzed by Western blotting and ECL using monoclonal antibodies specific for actin, CA, and MA.

The resulting intracellular distribution of viral MA and CA proteins, in the absence of anti-CD147 mAb, was consistent with those in the prior art cited herein, *supra*. That is, 1.5 hours after infection, viral MA and CA proteins underwent characteristic translocations from the membrane (M), into the cytoskeleton (CS), and cytosol fractions, respectively (Figure 7E,

5    isotype panel, lanes "CS"and "C," respectively).

However, no such protein translocation was observed in MT-4 cells treated with anti-CD147 mAb (UM-8D6), wherein both MA and CA remained associated with the cell-membrane fraction (Figure 7E, α-CD147 panel, lane "M"). Therefore, in the presence of anti-CD147 mAb (UM-8D6), virus was retained at the cell membrane, indicating that EMMPRIN is involved in

10   regulation of the HIV-1 uncoating step during infection.


## Example 7

### Viral Resistance to the Inhibitory Effect of Anti-CD147 mAb Correlated with Resistance to CsA

15   This example provides *in vitro* experiments and assays showing that HIV resistance to the inhibitory effect of anti-CD147 mAb correlated with resistance to CsA, indicating that the antibody interfered with a CyPA-dependent step in virus infection. Specifically, replication of A224E (a CsA-resistant, CA mutant HIV strain) in human PBMC was resistant to treatment with anti-CD147 mAb (UM-8D6) (Figure 8).

20   *Methods. Co-culture protocol for de novo HIV infection.* 293T cells were transfected with molecular clones of isogenic wild-type or A224E CA mutant viruses, and cultured for 3 days in the presence or absence of CsA (1 μM). The cells were washed on day 3 post-transfection and either cultured for an additional 4 days without CsA, or used for co-culture with PHA-activated CD8+ T cell-depleted PBMC (20 blood mononuclear cells per one 293T cell) in

25   the presence or absence of anti-CD147 mAb (UM-8D6; at 100 μg/ml) but without addition of CsA (to avoid virus-unrelated immunosuppressive effects of the drug on T cells that affected replication of both viruses). After 4 days of co-cultivation, virus replication was assayed by reverse transcriptase (RT) activity in culture supernatants using LTR U/5 primers [SEQ ID NO:6] and [SEQ ID NO:7], and probe [SEQ ID NO:8] as described herein above under

30   "Methods," Example 6.

*Replication of A224E (a CsA-resistant, CA mutant HIV strain) in human PBMC was resistant to treatment with anti-CD147 mAb.* CsA interacts with CyPA, and has been shown to thereby interfere with Gag (CA antigen portion)-CyPA interactions *in vitro*, block cyclophilin incorporation into virions, and inhibit replication of HIV-1 in cell culture. *See* summary herein

35   under "Background," *supra*. These data are consistent with a model whereby the interaction of Gag with CyPA is necessary for the formation of infectious HIV-1 virions (Sherry et al., *Proc. Natl. Acad. Sci. U.S.A.* 95:1758-1763, 1998). Accordingly, infection by CsA-resistant HIV strains (*e.g.*, strains with CA mutations that confer CsA resistance, a CyPA-dependant trait) may

reasonably be expected to be either not dependent on CyPA, or differentially affected by CyPA receptor antagonists.

Therefore, infection by the A224E virus strain, that harbors a CA mutation that confers CsA resistance, was analyzed to determine whether the inhibitory effect of anti-CD147 mAb

5  correlates with resistance to CsA. Such a correlation would be additional evidence that the anti-CD147 antibody interferes with a CyPA-dependent step in virus infection.

293T cells were transfected with molecular clones of HIV-1$_{NLA-3}$ ("WT") (Figure 8, left panel) or mutant A224E (Figure 8, right panel) viruses in the absence ("C") or presence ("CsA") of cyclosporin A (1 μM) as described herein above in this Example under "Methods." Virus-

10  producing transfected cells were washed and cultured in quadruplicate for an additional 4 days with ("293T + CD4$^+$ T cells") or without ("293T") addition of CD8$^+$ T cell-depleted PBMC and anti-CD147 mAb (UM-8D6; at 100 μg/ml). Virus replication was assayed by measuring RT activity in culture supernatants as described above under "Methods.". Results are presented as mean ± SE.

15  The amount of virus produced in this system reflects the efficiency of *de novo* infection of T cells rather than accumulation of the virus produced from transfected 293T cells. Anti-CD147 mAb (UM-8D6) did not inhibit replication of the CsA-resistant A224E virus, whereas replication of the wild-type virus was significantly diminished. A224E virus collected from CsA-treated 293T cells replicated to levels similar to those of the virus collected from untreated

20  cells, consistent with its CsA-resistant phenotype (Braaten et al., *J. Virol.* 70:5170-5176, 1996), whereas wild-type virus collected in the presence of CsA was significantly attenuated.

Therefore, viral resistance to the inhibitory effect of anti-CD147 mAb (UM-8D6) correlated with resistance to CsA. These data provide additional evidence that the antibody interferes with a CyPA-dependent step in virus infection.

25

## Example 8

**Peptides Derived from the Transmembrane Region of EMMPRIN Substantially Inhibited Infection of CHO-EMM Cells and Human PBMC by Pseudotyped Retroviruses Carrying the HIV-1 Core**

30  This example provides *in vitro* experiments and assays showing that peptides derived from the transmembrane region of the EMMPRIN protein inhibit HIV-1 infection. Specifically, an eight-amino acid polypeptide ([SEQ ID NO:3]) corresponding in sequence to a region at the amino-terminal end of the EMMPRIN transmembrane domain (*see* [SEQ ID NO:2]) blocked infection of CHO-EMM cells (Figure 9) and human PBMC by Env$^{MuLV}$- and Env$^{ADA}$-

35  pseudotyped luc-HIV-1 viruses, respectively.

*Methods.* CHO cell lines, infection of CHO cells with Env$^{MuLV}$-pseudotyped luc-HIV-1 variants and single-cycle luciferase assays were as described herein above under "Methods," Example 5. Preparation and infection of PHA-activated human PBMC with Env$^{ADA}$-

pseudotyped luc-HIV-1 was as described herein above under "Methods," Example 5 and Example 6. PBMC Lucifererase assays were performed as described for CHO cell lines.

        *Peptides.* Peptides 1 [SEQ ID NO:3], 2 [SEQ ID NO:4] and scrambled peptide 1 [SEQ ID NO:5] were obtained as custom polypeptides from Research Genetics (Huntsville, Alabama).

5        ***Peptide derived from the transmembrane region of CD147 inhibits MuLV-pseudotyped HIV-1 infection of CHO-EMM cells.*** The EMMPRIN transmembrane domain corresponds to amino acids 204-228 of the EMMPRIN protein sequence [SEQ ID NO:2]. In view of the novel EMMPRIN/CyP interaction of the present invention, peptides corresponding to sub-regions within the EMMPRIN transmembrane region were prepared to determine whether they could

10      inhibit HIV-1 infection. Peptide 1 (LAALWPFL) is an eight-amino acid sequence [SEQ ID NO:3] corresponding to amino acids 206-213 of EMMPRIN. Peptide 2 (GIVAEVLVL) is a nine-amino acid polypeptide sequence [SEQ ID NO:4] corresponding to amino acids 214-222 of EMMPRIN. "Scrambled peptide 1" (FAWPLLLA), the randomized sequence control, was made according to a randomized sequence of the amino acid residues of peptide 1.

15      CHO and CHO-EMM ("CHO.CD147") cells were infected as described above with recombinant luciferase-expressing HIV-1 pseudotyped with MuLV envelop in the presence of the indicated concentrations of peptides 1, 2, or scrambled peptide 1, or with no peptide. Luciferase expression was measured on day 4 post-infection using a luminometer.

        · Figure 9 shows that MuLV-psueudotyped HIV-1 infection of CHO-EMM cells was

20      substantially and specifically inhibited by peptide 1 [SEQ ID NO:3] in a concentration-dependent manner. HIV-1 infection was essentially blocked in the presence of 1 nM peptide 1.  · Neither peptide 2, nor scrambled peptide 1 inhibited HIV-1 infection in this assay.

        ***Peptide derived from the transmembrane region of CD147 inhibits HIV-1 infection of human PBMC.*** PHA-activated human PBMC cultures were infected with Env$^{ADA}$-pseudotyped

25      luc-HIV-1 in the presence of the indicated concentrations of peptides 1, 2 or scrambled peptide 1. As described above for Env$^{MuLV}$-psueudotyped HIV-1 infection of CHO-EMM cells, Env$^{ADA}$-pseudotyped luc-HIV-1 infection of PBMC was essentially blocked in the presence of 1 nM peptide 1. Neither peptide 2, nor scrambled peptide 1 inhibited Env$^{ADA}$-pseudotyped luc-HIV-1 infection in this assay.

30      ***EMMPRIN transmembrane polypeptides antagonize the CyP/EMMPRIN interaction.*** The above-identified activity of peptide 1 ([SEQ ID NO:3]) in inhibiting infection of CHO-EMM cells and PBMC by Env$^{MuLV}$- and Env$^{ADA}$-pseudotyped luc-HIV-1, respectively, is relevant to the interpretation of data presented herein above under "Example 1," regarding the retention of EMMPRIN by CyPA-coated sepharose beads. Under Example 1, the specific *in vitro* binding

35      interaction between EMMPRIN and CyPA was observed only with the full-length EMMPRIN (containing ecd, tm and icd domains) protein, and not with EMMPRIN lacking the tm and icd domains. As stated under Example 1, this result either reflected a requirement for the EMMPRIN transmembrane and/or intracellular domains for proper presentation of an ecd-CyP

binding site to CyPA on the sepharose beads, or was consistent with a mechanism whereby, CyPA directly interacted with the tm domain, with either ecd or tm domains or with both domains.

5      Without being bound by any specific mechanism, the above-identified inhibition of HIV-1 infection by EMMPRIN transmembrane peptide 1 is consistent with mechanism for CyP/EMMPRIN interaction whereby CyP directly interacts with a region of the EMMPRIN transmembrane domain, and where said interaction was effectively antagonized by peptide 1 ([SEQ ID NO:3]) on both CHO-EMM cells and PBMC.

10     **In summary:** Agents that modify the CyP/EMMPRIN binding interaction have utility for the treatment or prevention of conditions characterized by local or systemic CyP release, synthesis or binding such as HIV-1 infection, AIDS and AIDS-related disorders, RA and Cancer.

*Rheumatoid Arthritis (RA)*. The examples and embodiments of the present invention show that CyP interacts with, and specifically binds to the previously known and broadly

15     distributed cell-surface protein, EMMPRIN. Significantly, EMMPRIN had no known ligand prior to the present invention, and was believed only to be a potential adhesion molecule capable of mediating (*i.e.*, acting as a cell-surface "ligand") cell-cell interactions.

Additionally, examples and embodiments of the present invention demonstrate that CyP induces signal transduction in CHO-EMM cells that constitutively express EMMPRIN on their

20     surface. This response was shown to be specific, because it was blocked by the addition of anti-EMMPRIN specific antibodies. Moreover, the tyrosine kinase inhibitor Genistein completely abrogated CyP-induced $Ca^{2+}$ mobilization in CHO-EMM cells, indicating that EMMPRIN-mediated $Ca^{2+}$ mobilization occurs through known intracellular signal transduction pathways.

Furthermore, treatment of cultured human primary lung fibroblasts with CyP induced the

25     release of proMMP-1 and enhanced the production of proMMP-3. These two matrix metalloproteinases are known to be upregulated in synovial fluid and tissues in patients with RA (McCachren, *Arthritis Rheum.* 34:1085-1093, 1991; Keyszer et al., *J. Rheumatol.* 26:251-258, 1991; Keysze, et al., *Z. Rheumatology* 57:392-398, 1998; Matsuyama, *Pediatr. Int.* 41:239-245, 1999).

30     The examples and embodiments of the present invention, interpreted in view of previous demonstrations by the present inventors and others that CyPA is chemotactic for neutrophils (Sherry et al., *Proc. Natl. Acad. Sci. USA* 89:3511-3515, 1992; Xu et al., *J. Biol. Chem.* 267:11968-11971, 1992; Leiva & Lyttle, *Biochem. Biophys. Res. Commun.* 186:1178-1183, 1992), and that CyPA is markedly up-regulated in the joints of patients with active RA disease

35     and correlates with RA disease severity (Billich et al., *J. Exp. Med.* 185:975-980, 1997), shows that CyP mediates some of the pathology associated with rheumatoid arthritis via binding to, and signaling through EMMPRIN that is expressed on neutrophils and fibroblasts present in the RA joint.

Blocking this interaction will suppress the infiltration of neutrophils into the inflamed joint, and decrease the amount of proMMP-1 and proMMP-3 released into the synovium, thereby decreasing disease-associated pathology, and ultimately providing benefit to the patient.

*HIV-1 Infection and Acquired Immune Deficiency Syndrome (AIDS).* The examples
5   and embodiments of the present invention also provide EMMPRIN as an additional and novel molecular participant, and thus a novel therapeutic intervention target in the complex interactions between viral and cellular proteins during HIV-1 infection. Specifically, the anti-CD147 *(i.e.,* anti- EMMPRIN) mAb (UM-8D6) blocked a step in HIV-1 replication following virus-cell fusion but preceding formation of the functional pre-integration complex (PIC) that
10  supports reverse transcription.

Without being limited to a particular theory, the most likely mechanism consistent with the present invention is that EMMPRIN facilitates virus uncoating and translocation into the subcellular compartment *(i.e.,* cytoskeleton) where reverse transcription can take place. Significantly, anti-CD147 (UM-8D6) did not inhibit replication of a CsA-resistant HIV-1 mutant
15  (A224E, carrying a CA protein mutation) that displays CyPA-independent infection, further proving that the antibody interferes with a CyPA-dependent step in virus infection.

Thus, the HIV virus commandeers the whole CyPA/EMMPRIN interaction pathway for its purposes.

*Cancer and Connective Tissue Diseases.* The present invention also provides a method
20  of treatment for cancer and many connective tissue diseases. Such a utility is supported by (1) the fact that EMMPRIN-expressing tumor cells have been shown to up-regulate the expression of MMPs in fibroblasts co-cultured therewith (Biswas et al., *Cancer Res.* 55:434-439, 1995), (2) CyP activated and/or enhanced MMP production in human fibroblasts, as disclosed herein, and (3) the deregulated action of MMPs contributes to the pathological destruction of the
25  extracellular matrix in many connective tissue diseases including, *e.g.,* arthritis, periodontitis, tissue ulceration, and in cancer cell invasion and metastasis (Kahariet al., *Exp. Dermatol.* 6:199-213, 1997; Keyszer et al., *J. Rheumatol.* 26:251-258, 1999; Benbow et al., *J. Biol. Chem.* 274:25371-25378, 1999; Keyszer et al., *Z. Rheumatology* 57:392-398, 1998).

SEQUENCE LISTING

(1)     GENERAL INFORMATION:
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         (vi)     CURRENT APPLICATION DATA:
                  (A)    APPLICATION NUMBER:   to be assigned
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(2)     INFORMATION FOR SEQ ID NO:1:
         (i)      SEQUENCE CHARACTERISTICS:
                  (A)    LENGTH: 1490 base pairs
                  (B)    TYPE: nucleic acid
                  (C)    STRANDEDNESS:
                  (D)    TOPOLOGY: linear
         (ii)     MOLECULE TYPE: cDNA to mRNA
         (iii)    HYPOTHETICAL: no
         (iv)     ANTI-SENSE: no
         (vi)     ORIGINAL SOURCE: human
                  (A)    ORGANISM: Homo sapiens
         (ix)     FEATURE:

58

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                    (A)    NAME/KEY:  CDS
                    (B)    LOCATION:  nucleotides 46-855
                    (A)    NAME/KEY:  sig. peptide
                    (B)    LOCATION:  nucleotides 46-108
  5                 (A)    NAME/KEY:  extracellular domain
                    (B)    LOCATION:  nucleotides 109-660
                    (A)    NAME/KEY:  transmembrane domain
                    (B)    LOCATION:  nucleotides 661-732
                    (A)    NAME/KEY:  cytoplasmic domain
 10                 (B)    LOCATION:  nucleotides 733-852
            (x)     PUBLICATION INFORMATION:
                    (A)    AUTHORS: DeCastro et al.
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      EMMPRIN, an extracellular matrix metalloproteinase inducer
 15                 (C)    JOURNAL: J. Invest. Dermatol.
                    (D)    VOLUME: 106
                    (E)    ISSUE: 6
                    (F)    PAGES: 1260-1265
                    (G)    DATE: 1996
 20                 (K)    RELEVANT RESIDUES IN SEQ ID NO:1: FROM 46
      TO 852
            (xi)    SEQUENCE DESCRIPTION: SEQ ID NO:1:
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                                                     Met Ala
                                                      1

GCT GCG CTG TTC GTG CTG CTG GGA TTC GCG CTG CTG GGC ACC CAC      96
Ala Ala Leu Phe Val Leu Leu Gly Phe Ala Leu Leu Gly Thr His
         5                   10                  15

GGA GGC TCC GGG GCT GCT GGC ACA GTC TTC ACT ACC GTA GAA GAC      141
Gly Ala Ser Gly Ala Ala Gly Thr Val Phe Thr Thr Val Glu Asp
             20                  25                  30

CTT GGC TCC AAG ATA CTC CTC ACC TGC TCC TTG AAT GAC AGC GCC      186
Leu Gly Ser Lys Ile Leu Leu Thr Cys Ser Leu Asn Asp Ser Ala
                 35                  40                  45

ACA GAG GTC ACA GGG CAC CGC TGG CTG AAG GGG GGC GTG GTG CTG      231
Thr Glu Val Thr Gly His Arg Trp Leu Lys Gly Gly Val Val Leu
                 50                  55                  60

AAG GAG GAC GCG CTG CCC GGC CAG AAA ACG GAG TTC AAG GTG GAC      276
Lys Glu Asp Ala Leu Pro Gly Gln Lys Thr Glu Phe Lys Val Asp
             65                  70                  75

TCC GAC GAC CAG TGG GGA GAG TAC TCC TGC GTC TTC CTC CCC GAG   321
Ser Asp Asp Gln Trp Gly Glu Tyr Ser Cys Val Phe Leu Pro Glu
        80                      85                  90

5   CCC ATG GGC ACG GCC AAC ATC CAG CTC CAC GGG CCT CCC AGA GTG   366
Pro Met Gly Thr Ala Asn Ile Gln Leu His Gly Pro Pro Arg Val
        95                      100                 105

AAG GCC GTG AAG TCG TCA GAA CAC ATC AAC GAG GGG GAG ACG GCC   411
10  Lys Ala Val Lys Ser Ser Glu His Ile Asn Glu Gly Glu Thr Ala
        110                     115                 120

ATG CTG GTC TGC AAG TCA GAG TCC GTG CCA CCT GTC ACT GAC TGG   456
Met Leu Val Cys Lys Ser Glu Ser Val Pro Pro Val Thr Asp Trp
15      125                     130                 135

GCC TGG TAC AAG ATC ACT GAC TCT GAG GAC AAG GCC CTC ATG AAC   501
Ala Trp Tyr Lys Ile Thr Asp Ser Glu Asp Lys Ala Leu Met Asn
        140                     145                 150
20
GGC TCC GAG AGC AGG TTC TTC GTG AGT TCC TCG CAG GGC CGG TCA   546
Gly Ser Glu Ser Arg Phe Phe Val Ser Ser Ser Gln Gly Arg Ser
        155                     160                 165

25  GAG CTA CAC ATT GAG AAC CTG AAC ATG GAG GCC GAT CCC GGC CAG   591
Glu Leu His Ile Glu Asn Leu Asn Met Glu Ala Asp Pro Gly Gln
        170                     175                 180

TAC CGG TGC AAC GGC ACC AGC TCC AAG GGC TCC GAC CAG GCC ATC   636
30  Tyr Arg Cys Asn Gly Thr Ser Ser Lys Gly Ser Asp Gln Ala Ile
        185                     190                 195

ATC ACG CTC CGC GTG CGC AGC CAC CTG GCC GCC CTC TGG CCC TTC   681
Ile Thr Leu Arg Val Arg Ser His Leu Ala Ala Leu Trp Pro Phe
35      200                     205                 210

CTG GGC ATC GTG GCT GAG GTG CTG GTG CTG GTC ACC ATC ATC TTC   726
Leu Gly Ile Val Ala Glu Val Leu Val Leu Val Thr Ile Ile Phe
        215                     220                 225
40
ATC TAC GAG AAG CGC CGG AAG CCC GAG GAC GTC CTG GAT GAT GAC   771
Ile Tyr Glu Lys Arg Arg Lys Pro Glu Asp Val Leu Asp Asp Asp
        230                     235                 240

45  GAC GCC GGC TCT GCA CCC CTG AAG AGC AGC GGG CAG CAC CAG AAT   816
Asp Ala Gly Ser Ala Pro Leu Lys Ser Ser Gly Gln His Gln Asn
        245                     250                 255

60

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GAC AAA GGC AAG AAC GTC CGC CAG AGG AAC TCT TCC TGAGGCAGGT    862
Asp Lys Gly Lys Asn Val Arg Gln Arg Asn Ser Ser
        260                 265
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5

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GGCCCGAGGA CGCTCCCTGC TCCGCGTCTG CGCCGCCGCC GGAGTCCACT        912

CCCAGTGCTT GCAAGATTCC AAGTTCTCAC CTCTTAAAGA AAACCCACCC        962
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10
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CGTAGATTCC CATCATACAC TTCCTTCTTT TTTAAAAAAG TTGGGTTTTC        1012

TCCATTCAGG ATTCTGTTCC TTAGGATTTT TTCCTTCTGA AGTGTTTCAC        1062

GAGAGCCCGG GAGCTGCTGC CCTGCGGCCC CGTCTGTGGC TTTCAGCCTC        1112
```
15
```
TGGGTCTGAG TCATGGCCGG GTGGGCGGCA CAGCCTTCTC CACTGGCCGG        1162

AGTCAGTGCC AGGTCCTTGC CCTTTGTGGA AAGTCACAGG TCACACGAGG        1212
```
20
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GGCCCCGTGT CCTGCCTGTC TGAAGCCAAT GCTGTCTGGT TGCGCCATTT        1262

TTGTGCTTTT ATGTTTAATT TTATGAGGGC CACGGGTCTG TGTTCGACTC        1312
```
```
AGCCTCAGGG ACGACTCTGA CCTCTTGGCC ACAGAGGACT CAACTTGCCC        1362
```
25
```
ACACCGAGGG CGACCCCGTC ACAGCCTCAA GTCACTCCCA AGCCCCCTCC        1412

TTGTCTGTGC ATCCGGGGGC AGCTCTGGAG GGGGTTTGCT GGGGAACTGG        1462
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30
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CGCCATCGCC GGGACTCCAG AACCGCCG                                1490
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    (2)     INFORMATION FOR SEQ ID NO:2:
            (i)     SEQUENCE CHARACTERISTICS:
35                          (A)    LENGTH: 269
                            (B)    TYPE: amino acid
                            (C)    STRANDEDNESS: single
                            (D)    TOPOLOGY: linear
            (ii)    MOLECULE TYPE: polypeptide
40          (iii)   HYPOTHETICAL: no
            (iv)    ANTI-SENSE: no
            (vi)    ORIGINAL SOURCE: human
                            (A)    ORGANISM: Homo sapiens
            (ix)    FEATURE:
45                          (A)    NAME/KEY: Protein
                            (B)    LOCATION: residues 1-269
                            (A)    NAME/KEY: sig. peptide
```

(B)     LOCATION: residues 1-21
(A)     NAME/KEY: extracellular domain
(B)     LOCATION: residues 22-205
(A)     NAME/KEY: transmembrane domain
(B)     LOCATION: residues 206-229
(A)     NAME/KEY: cytoplasmic domain
(B)     LOCATION: residues 230-269
(x)     PUBLICATION INFORMATION:
(A)     AUTHORS: DeCastro et al.
(B)     TITLE: Human keratinocytes express
EMMPRIN, an extracellular matrix metalloproteinase inducer
(C)     JOURNAL: J. Invest. Dermatol.
(D)     VOLUME: 106
(E)     ISSUE: 6
(F)     PAGES: 1260-1265
(G)     DATE: 1996
(K)     RELEVANT RESIDUES IN SEQ ID NO:2: FROM 1
TO 269
(xi)    SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Ala Ala Leu Phe Val Leu Leu Gly Phe Ala Leu Leu Gly Thr
                 5                  10                  15

His Gly Ala Ser Gly Ala Ala Gly Thr Val Phe Thr Thr Val Glu Asp
             20                  25                  30

Leu Gly Ser Lys Ile Leu Leu Thr Cys Ser Leu Asn Asp Ser Ala Thr
             35                  40                  45

Glu Val Thr Gly His Arg Trp Leu Lys Gly Gly Val Val Leu Lys Glu
         50                  55                  60

Asp Ala Leu Pro Gly Gln Lys Thr Glu Phe Lys Val Asp Ser Asp Asp
     65              70.                 75                  80

Gln Trp Gly Glu Tyr Ser Cys Val Phe Leu Pro Glu Pro Met Gly Thr
                 85                  90                  95

Ala Asn Ile Gln Leu His Gly Pro Pro Arg Val Lys Ala Val Lys Ser
             100                 105                 110

Ser Glu His Ile Asn Glu Gly Glu Thr Ala Met Leu Val Cys Lys Ser
             115                 120                 125

Glu Ser Val Pro Pro Val Thr Asp Trp Ala Trp Tyr Lys Ile Thr Asp
         130                 135                 140

Ser Glu Asp Lys Ala Leu Met Asn Gly Ser Glu Ser Arg Phe Phe Val
145                 150                 155                 160

Ser Ser Ser Gln Gly Arg Ser Glu Leu His Ile Glu Asn Leu Asn Met
                165                 170                 175

Glu Ala Asp Pro Gly Gln Tyr Arg Cys Asn Gly Thr Ser Ser Lys Gly
            180                 185                 190

Ser Asp Gln Ala Ile Ile Thr Leu Arg Val Arg Ser His Leu Ala Ala
        195                 200                 205

Leu Trp Pro Phe Leu Gly Ile Val Ala Glu Val Leu Val Leu Val Thr
    210                 215                 220

Ile Ile Phe Ile Tyr Glu Lys Arg Arg Lys Pro Glu Asp Val Leu Asp
225                 230                 235                 240

Asp Asp Asp Ala Gly Ser Ala Pro Leu Lys Ser Ser Gly Gln His Gln
                235                 250                 255

Asn Asp Lys Gly Lys Asn Val Arg Gln Arg Asn Ser Ser
            260                 265

(2)     INFORMATION FOR SEQ ID NO:3:
        (i)     SEQUENCE CHARACTERISTICS:
                (A)     LENGTH: 8
                (B)     TYPE: amino acid
                (C)     STRANDEDNESS: single
                (D)     TOPOLOGY: linear
        (ii)    MOLECULE TYPE: polypeptide
        (iii)   HYPOTHETICAL: no
        (iv)    ANTI-SENSE: no
        (vi)    ORIGINAL SOURCE: human
                (A)     ORGANISM: Homo sapiens
        (xi)    SEQUENCE DESCRIPTION: SEQ ID NO:3:

Leu Ala Ala Leu Trp Pro Phe Leu
                5

(2)     INFORMATION FOR SEQ ID NO:4:
        (i)     SEQUENCE CHARACTERISTICS:
                (A)     LENGTH: 9
                (B)     TYPE: amino acid
                (C)     STRANDEDNESS: single

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                    (D)   TOPOLOGY: linear
           (ii)     MOLECULE TYPE: polypeptide
           (iii)    HYPOTHETICAL: no
           (iv)     ANTI-SENSE: no
     5     (vi)     ORIGINAL SOURCE: human
                    (A)   ORGANISM: Homo sapiens
           (xi)     SEQUENCE DESCRIPTION: SEQ ID NO:4:


   Gly Ile Val Ala Glu Val Leu Val Leu
    10                  5



     (2)      INFORMATION FOR SEQ ID NO:5:
              (i)     SEQUENCE CHARACTERISTICS:
    15                  (A)   LENGTH: 8
                        (B)   TYPE: amino acid
                        (C)   STRANDEDNESS: single
                        (D)   TOPOLOGY: linear
              (ii)     MOLECULE TYPE: polypeptide
    20        (iii)    HYPOTHETICAL: no
              (iv)     ANTI-SENSE: no
              (xi)     SEQUENCE DESCRIPTION: SEQ ID NO:5:


   Phe Ala Trp Pro Leu Leu Leu Ala
    25                  5



     (2)      INFORMATION FOR SEQ ID NO:6:
              (i)     SEQUENCE CHARACTERISTICS:
    30                  (A)   LENGTH: 21 nucleotides
                        (B)   TYPE: nucleic acid
                        (C)   STRANDEDNESS: single
                        (D)   TOPOLOGY: linear
              (ii)     MOLECULE TYPE: DNA oligonucleotide
    35        (iii)    HYPOTHETICAL: no
              (iv)     ANTI-SENSE: no
              (vi)     ORIGINAL SOURCE:
                        (A)   ORGANISM: HIV-1
              (ix)     FEATURE:
    40                  (A)   NAME/KEY: HIV-1 LTR sequence
                        (D)   OTHER INFORMATION: R/U5 sense primer
              (x)      PUBLICATION INFORMATION:
                        (A)   AUTHORS: Schmidtmayerova et al.
                        (B)   TITLE: Human immunodeficiency virus type
    45  1 T-lymphotropic strains enter macrophages via a CD4- and CXCR4-
    mediated pathway: replication is restricted at a postentry level
                        (C)   JOURNAL: J. Virol.
```

                           (D)   VOLUME: 72
                           (E)   PAGES: 4633-4642
                           (F)   DATE: 1998
                   (xi)   SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGCTAACTAG GGAACCCACT G          21


    (2)     INFORMATION FOR SEQ ID NO:7:
            (i)     SEQUENCE CHARACTERISTICS:
                           (A)   LENGTH: 24 nucleotides
                           (B)   TYPE: nucleic acid
                           (C)   STRANDEDNESS: single
                           (D)   TOPOLOGY: linear
            (ii)    MOLECULE TYPE: DNA oligonucleotide
            (iii)   HYPOTHETICAL: no
            (iv)    ANTI-SENSE: yes
            (vi)    ORIGINAL SOURCE:
                           (A)   ORGANISM: HIV-1
            (ix)    FEATURE:
                           (A)   NAME/KEY: HIV-1 LTR sequence
                           (D)   OTHER INFORMATION: R/U5 antisense primer
            (x)     PUBLICATION INFORMATION:
                           (A)   AUTHORS: Schmidtmayerova et al.
                           (B)   TITLE: Human immunodeficiency virus type
1 T-lymphotropic strains enter macrophages via a CD4- and CXCR4-
mediated pathway: replication is restricted at a postentry level
                           (C)   JOURNAL: J. Virol.
                           (D)   VOLUME: 72
                           (E)   PAGES: 4633-4642
                           (F)   DATE: 1998
            (xi)    SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTGCTAGAGA TTTTCCACAC TGAC      24


    (2)     INFORMATION FOR SEQ ID NO:8:
            (i)     SEQUENCE CHARACTERISTICS:
                           (A)   LENGTH: 21 nucleotides
                           (B)   TYPE: nucleic acid
                           (C)   STRANDEDNESS: single
                           (D)   TOPOLOGY: linear
            (ii)    MOLECULE TYPE: DNA oligonucleotide
            (iii)   HYPOTHETICAL: no
            (iv)    ANTI-SENSE:
            (vi)    ORIGINAL SOURCE:
                           (A)   ORGANISM: HIV-1

        (ix)    FEATURE:
                (A)    NAME/KEY: HIV-1 LTR sequence
                (D)    OTHER INFORMATION: R/U5 sequence probe
        (x)     PUBLICATION INFORMATION:
5               (A)    AUTHORS: Schmidtmayerova et al.
                (B)    TITLE: Human immunodeficiency virus type
1 T-lymphotropic strains enter macrophages via a CD4- and CXCR4-
mediated pathway: replication is restricted at a postentry level
                (C)    JOURNAL: *J. Virol.*
10              (D)    VOLUME: 72
                (E)    PAGES: 4633-4642
                (F)    DATE: 1998
        (xi)    SEQUENCE DESCRIPTION: SEQ ID NO:8:


15  TGTGTGCCCG TCTGTTGTGT G            21



    (2)     INFORMATION FOR SEQ ID NO:9:
        (i)     SEQUENCE CHARACTERISTICS:
20              (A)    LENGTH: 21 nucleotides
                (B)    TYPE: nucleic acid
                (C)    STRANDEDNESS: single
                (D)    TOPOLOGY: linear
        (ii)    MOLECULE TYPE: DNA oligonucleotide
25      (iii)   HYPOTHETICAL: no
        (iv)    ANTI-SENSE: no
        (vi)    ORIGINAL SOURCE:
                (A)    ORGANISM: HIV-1
        (ix)    FEATURE:
30              (A)    NAME/KEY: HIV-1 LTR sequence
                (D)    OTHER INFORMATION: LTR/gag sense primer
        (x)     PUBLICATION INFORMATION:
                (A)    AUTHORS: Schmidtmayerova et al.
                (B)    TITLE: Human immunodeficiency virus type
35  1 T-lymphotropic strains enter macrophages via a CD4- and CXCR4-
mediated pathway: replication is restricted at a postentry level
                (C)    JOURNAL: *J. Virol.*
                (D)    VOLUME: 72
                (E)    PAGES: 4633-4642
40              (F)    DATE: 1998
        (xi)    SEQUENCE DESCRIPTION: SEQ ID NO:9:


    CAGATATCCA CTGACCTTTG G            21


45

    (2)     INFORMATION FOR SEQ ID NO:10:
        (i)     SEQUENCE CHARACTERISTICS:

66

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                    (A)   LENGTH: 22 nucleotides
                    (B)   TYPE: nucleic acid
                    (C)   STRANDEDNESS: single
                    (D)   TOPOLOGY: linear
5       (ii)    MOLECULE TYPE: DNA oligonucleotide
        (iii)   HYPOTHETICAL: no
        (iv)    ANTI-SENSE: yes
        (vi)    ORIGINAL SOURCE:
                    (A)   ORGANISM: HIV-1
10      (ix)    FEATURE:
                    (A)   NAME/KEY: HIV-1 LTR sequence
                    (D)   OTHER INFORMATION: LTR/gag antisense
primer
        (x)     PUBLICATION INFORMATION:
15                  (A)   AUTHORS: Schmidtmayerova et al.
                    (B)   TITLE: Human immunodeficiency virus type
1 T-lymphotropic strains enter macrophages via a CD4- and CXCR4-
mediated pathway: replication is restricted at a postentry level
                    (C)   JOURNAL: J. Virol.
20                  (D)   VOLUME: 72
                    (E)   PAGES: 4633-4642
                    (F)   DATE: 1998
        (xi)    SEQUENCE DESCRIPTION: SEQ ID NO:10:


25  GCTTAATACT GACGCTCTCG CA          22



    (2)     INFORMATION FOR SEQ ID NO:11:
            (i)     SEQUENCE CHARACTERISTICS:
30                  (A)   LENGTH: 20 nucleotides
                    (B)   TYPE: nucleic acid
                    (C)   STRANDEDNESS: single
                    (D)   TOPOLOGY: linear
            (ii)    MOLECULE TYPE: DNA oligonucleotide
35          (iii)   HYPOTHETICAL: no
            (iv)    ANTI-SENSE:
            (vi)    ORIGINAL SOURCE:
                    (A)   ORGANISM: HIV-1
            (ix)    FEATURE:
40                  (A)   NAME/KEY: HIV-1 LTR sequence
                    (D)   OTHER INFORMATION: LTR/gag sequence probe
            (x)     PUBLICATION INFORMATION:
                    (A)   AUTHORS: Schmidtmayerova et al.
                    (B)   TITLE: Human immunodeficiency virus type
45  1 T-lymphotropic strains enter macrophages via a CD4- and CXCR4-
mediated pathway: replication is restricted at a postentry level
                    (C)   JOURNAL: J. Virol.
```

        (D)    VOLUME: 72

        (E)    PAGES: 4633-4642-

        (F)    DATE: 1998

    (xi)    SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAGGCTTAAG CAGTGGGTTC            20

We claim:

1. A method for treating or preventing inflammatory disease, autoimmune disease, tissue ulceration, tumor growth or metastasis or any condition characterized by local or systemic CyP release, synthesis or binding in a patient needing treatment, comprising administering to the patient a therapeutically effective amount of a CyP/EMMPRIN antagonist or agonist, wherein the CyP/EMMPRIN antagonist or agonist is selected from the group consisting of an anti-EMMPRIN antibody, a soluble EMMPRIN protein or polypeptide, a CyP protein or polypeptide and combinations thereof.
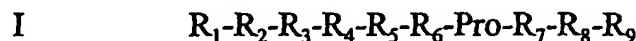
2. The method according to claim1 wherein the inflammatory or autoimmune disease is arthritis, EAE, ARDS or peridontitis.

3. The method according to claim 1 wherein the CyP/EMMPRIN antagonist or agonist is an anti-EMMPRIN antibody.

4. The method according to claim 3 wherein the anti-EMMPRIN antibody binds to the extracellular domain or the transmembrane domain of EMMPRIN.

5. The method according to claim 4 wherein the anti-EMMPRIN antibody binds to an epitope that is recognized by the UM-8D6 anti-CD147 monoclonal antibody.

6. The method according to claim 1 wherein the CyP/EMMPRIN antagonist or agonist is an EMMPRN polypeptide comprising an amino acid sequence of about 5 to 14 amino acids taken from a sequence extending from about residue 206 to about residue 229 of [SEQ ID NO:2] corresponding to the EMMPRIN transmembrane domain.

7. A method for treating or preventing inflammatory disease, autoimmune disease, tissue ulceration, tumor growth or metastasis or any condition characterized by local or systemic CyP release, synthesis or binding in a patient needing treatment, comprising administering to the patient a therapeutically effective amount of a compound of formula I wherein formula I is

$$I \qquad R_1\text{-}R_2\text{-}R_3\text{-}R_4\text{-}R_5\text{-}R_6\text{-}Pro\text{-}R_7\text{-}R_8\text{-}R_9$$

wherein: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$, $R_3$ and $R_4$ are independently Leu, Ile, Ala, Met or nothing; $R_5$ and $R_8$ are independently Leu, Ile, Ala or Met; $R_6$ and $R_7$ are independently Phe, Trp or Tyr; and -$R_9$ is -Gly, -GlyIle, -GlyIleVal or nothing.

8. The method according to claim 7 wherein, for formula I: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$ is Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$ is Phe; $R_8$ is Leu; and -$R_9$ is nothing.

9. The method according to claim 8 wherein, formula I: $R_1$- is nothing; $R_2$ is Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$ is Phe; $R_8$ is Leu; and -$R_9$ is nothing.

10. A method for decreasing the susceptibility of a cell to retroviral infection, wherein the cell expresses a functional EMMPRIN receptor, and wherein a retrovirus uses the EMMPRIN receptor in a pre-integration step of viral infection, comprising contacting the cell·

with an amount of a CyPA/EMMPRIN antagonist or agonist sufficient to inhibit the pre-integration step of retroviral infection, and wherein the CyPA/EMMPRIN antagonist or agonist is selected from the group consisting of an anti-EMMPRIN antibody, a soluble EMMPRIN protein or polypeptide, a CyPA protein or polypeptide and combinations thereof.

11.     The method according to claim 10 wherein the CyPA/EMMPRIN antagonist or agonist is an anti-EMMPRIN antibody.

12.     The method according to claim 11 wherein the anti-EMMPRIN antibody binds to the extracellular domain or the transmembrane domain of EMMPRIN.

13.     The method according to claim 12 wherein the anti-EMMPRIN antibody binds to an epitope recognized by the UM-8D6 anti-CD147 monoclonal antibody.

14.     The method according to claim 10 wherein the CyP/EMMPRIN antagonist or agonist is an EMMPRN polypeptide comprising an amino acid sequence of about 5 to 14 amino acids taken from a sequence extending from about residue 206 to about residue 229 of [SEQ ID NO:2] corresponding to the EMMPRIN transmembrane domain.

15.     A method for decreasing the susceptibility of a cell to retroviral infection, wherein the cell expresses a functional EMMPRIN receptor, and wherein a retrovirus uses the EMMPRIN receptor in a pre-integration step of viral infection, comprising contacting the cell with an amount of a compound of formula I sufficient to inhibit the pre-integration step of retroviral infection, wherein formula I is

$$ I \qquad R_1\text{-}R_2\text{-}R_3\text{-}R_4\text{-}R_5\text{-}R_6\text{-Pro-}R_7\text{-}R_8\text{-}R_9 $$

wherein: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$, $R_3$ and $R_4$ are independently Leu, Ile, Ala, Met or nothing; $R_5$ and $R_8$ are independently Leu, Ile, Ala or Met; $R_6$ and $R_7$ are independently Phe, Trp or Tyr; and -$R_9$ is -Gly, -GlyIle, -GlyIleVal or nothing.

16.     The method according to claim 15 wherein, for formula I: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$ is Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$ is Phe; $R_8$ is Leu; and -$R_9$ is nothing.

17.     The method according to claim 16 wherein, for formula I: $R_1$- is nothing; $R_2$ is Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$ is Phe; $R_8$ is Leu; and -$R_9$ is nothing.

18.     A method for treating or preventing HIV infection, AIDS or AIDS-related disorders in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a CyPA/EMMPRIN antagonist or agonist, wherein the CyPA/EMMPRIN antagonist or agonist is selected from the group consisting of an anti-EMMPRIN antibody, a soluble EMMPRIN protein or polypeptide, a CyPA protein or polypeptide and combinations thereof.

19.     The method according to claim 18 wherein the CyPA/EMMPRIN antagonist or agonist is an anti-EMMPRIN antibody.

20. The method according to claim 19 wherein the anti-EMMPRIN antibody binds to the extracellular domain or the transmembrane domain of EMMPRIN.

21. The method according to claim 20 wherein the anti-EMMPRIN antibody binds to an epitope recognized by the UM-8D6 anti-CD147 monoclonal antibody.

22. The method according to claim 18 wherein the CyP/EMMPRIN antagonist or agonist is an EMMPRN polypeptide comprising an amino acid sequence of about 5 to 14 amino acids taken from a sequence extending from about residue 206 to about residue 229 of [SEQ ID NO:2] corresponding to the EMMPRIN transmembrane domain.

23. A method for treating or preventing HIV infection, AIDS or AIDS-related disorders in a patient in need thereof comprising administering to the patient a therapeutically effective amount of a compound of formula I, wherein formula I is

$$I \qquad R_1\text{-}R_2\text{-}R_3\text{-}R_4\text{-}R_5\text{-}R_6\text{-Pro-}R_7\text{-}R_8\text{-}R_9$$
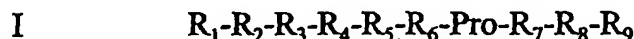
wherein: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$, $R_3$ and $R_4$ are independently Leu, Ile, Ala, Met or nothing; $R_5$ and $R_8$ are independently Leu, Ile, Ala or Met; $R_6$ and $R_7$ are independently Phe, Trp or Tyr; and -$R_9$ is -Gly, -GlyIle, -GlyIleVal or nothing.

24. The method according to claim 23 wherein, for formula I: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$ is Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$ is Phe; $R_8$ is Leu; and -$R_9$ is nothing.

25. The method according to claim 24 wherein, for formula I: $R_1$- is nothing; $R_2$ is Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$ is Phe; $R_8$ is Leu; and -$R_9$ is nothing.

26. The method of claims 3, 11 or 19 wherein the antibody is a monclonal antibody.

27. The method of claim 26 wherein the monoclonal antibody is a single-chain antibody, chimeric antibody, humanized antibody, or Fab fragment.

28. A method for identifying test compounds having therapeutic activity for HIV infection, AIDS or AIDS-related disorders, inflammatory disease, autoimmune disease, tissue ulceration, tumor growth or metastasis or any condition characterized by local or systemic CyP release, synthesis or binding in a patient needing treatment, comprising:

(a) contacting a test compound with a functional CyP protein and a functional EMMPRIN protein wherein at least one of the proteins bears a detectable label;

(b) assaying any resulting EMMPRIN/CyP complex for the presence of the label; and

(c) determining whether the test compound inhibited binding of the CyP protein to the EMMPRIN protein, whereby test compounds that inhibit binding of the CyP protein to the EMMPRIN protein are identified as therapeutic compounds.

29. The method according to claim 28 wherein either the functional EMMPRIN protein or the functional CyP protein is immobilized onto a solid phase.

30.     The method according to claim 28 wherein the CyP protein or the EMMPRIN protein is labeled with a radiolabel, a fluorescent reporter or quencher moiety, an enzymic label that catalyzes a colorometric or fluorometric change or combinations thereof.

31.     The method according to claim 28 wherein the inflammatory or autoimmune
5     disease is arthritis, EAE, ARDS or peridontitis.

32.     A method for identifying test compounds having therapeutic activity for HIV infection, AIDS or AIDS-related disorders, inflammatory disease, autoimmune disease, tumor growth or metastasis or any condition characterized by local or systemic CyP release, synthesis or binding in a patient needing treatment, comprising:

10          (a) contacting a test compound, in the presence of a functional CyP protein, with a cell expressing a functional EMMPRIN protein; and

            (b) determining whether the test compound inhibits binding of the CyP protein to the EMMPRIN protein, whereby test compounds that inhibit binding of the CyP protein to the EMMPRIN protein are identified as therapeutic compounds.

15      33.     The method according to claim 32 wherein the inflammatory or autoimmune disease is arthritis, EAE, ARDS or peridontitis.

34.     The method of claim 32 wherein the cell expresses recombinant EMMPRIN, CD4, CXCR4 or combinations thereof.

35.     The method of claim 32 wherein determination of the inhibition of binding of the
20     CyP protein to the EMMPRIN protein is based on an assay selected from the group consisting of CyP/EMMPRIN antagonist assays, receptor sensitization or desensitization assays, receptor up- or down-regulation assays, EMMPRIN-mediated signal transduction assays, $Ca^{2+}$ mobilization assays, matrix metalloproteinase expression or activity assays, cell growth rate assays, HIV-1 infection assays, and assays based on detection of a specific marker of cell cycle or cell
25     differentiation.

36.     The method of claim 35 wherein the EMMPRIN-mediated signal transduction assays are based on determining the phosphorylation or activation status of an intracellular protein, wherein the intracellular protein is selected from the group consisting of Ras, PKA, RAP1, B-Raf, Mek, and MAPK, whereby test compounds that alter the phosphorylation or
30     activation status of said proteins, relative to their phosphorylation or activation status in control cells, are identified as therapeutic compounds.

37.     The method of claim 35 wherein the specific marker of cell cycle or cell differentiation is selected from the group consisting of cell-cycle regulatory proteins cyclin D1, cyclin E and p21/Waf1, whereby test compounds that alter said cell-cycle markers, relative their
35     status in control cells, are identified as therapeutic compounds.

38.     The method of claim 35 wherein the matrix metalloproteinase expression assays measure the expression or activity of proMMP-1, proMMP-3, proMMP-9, MMP-1, MMP-3 or MMP-9.

39.     The method of claim 35 wherein the HIV-1 infection assays measure viral reverse transcriptase activity or virus-directed luciferase expression.

40.     A pharmaceutical composition comprising a compound of formula I or a pharmaceutically-acceptable salt thereof, and a pharmaceutically-acceptable carrier, wherein formula I is

I              $R_1$-$R_2$-$R_3$-$R_4$-$R_5$-$R_6$-Pro-$R_7$-$R_8$-$R_9$
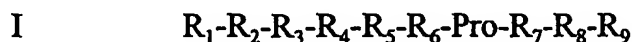
wherein: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$, $R_3$ and $R_4$ are independently Leu, Ile, Ala, Met or nothing; $R_5$ and $R_8$ are independently Leu, Ile, Ala or Met; $R_6$ and $R_7$ are independently Phe, Trp or Tyr; and -$R_9$ is -Gly, -GlyIle, -GlyIleVal or nothing.

41.     The pharmaceutical composition of claim 40 wherein for formula I: $R_1$- is His-, SerHis-, ArgSerHis-, Lys-, LysLys-, Arg- or nothing; $R_2$ is Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$ is Phe; $R_8$ is Leu; and -$R_9$ is nothing.

42.     The pharmaceutical composition of claim 41 wherein for formula I: $R_1$- is nothing; $R_2$ is Leu; $R_3$ is Ala; $R_4$ is Ala; $R_5$ is Leu; $R_6$ is Trp; $R_7$ is Phe; $R_8$ is Leu; and -$R_9$ is nothing.

FIG.1A

FIG.1B

FIG.2

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FIG.3

FIG.4

FIG.5

FIG.6C

FIG.6B

FIG.6A

FIG.7A

FIG.7B

FIG.7C

FIG.7D

FIG.7E

FIG.8

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FIG.9